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PRINCIPAL INVESTIGATOR: Jen-Tsan Ashley Chi

CONTRACTING ORGANIZATION: Duke University

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Jen-Tsan Ashley Chi

E-Mail: jentsan.chi@duke.edu

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Duke University
2200 W Main St., STE 710
Durham, NC 27705

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14. ABSTRACT

As breast tumors expand in size, they rapidly exceed the local oxygen supply. A limited oxygen supply (hypoxia) forces tumor glycolysis and produces large amount of lactic acid (lactic acidosis). The regions of hypoxia and lactic acidosis in tumors are resistant to most treatments and largely responsible for relapse. While the clinical relevance of these stresses is well recognized, they are usually not incorporated in the discovery and validation of tumor therapeutic targets. Thus, very few options currently exist to target the cells exposed to these stresses. Since targeting tumor stresses is expected to have significant clinical benefit, research in this area is an urgent and unmet need for the breast cancer. Using functional genomic screens, we have identified a set of protein kinases are contextually essential under hypoxia or lactic acidosis. We will first validate the inhibition of synthetic lethal kinases to eradicate breast cancer cells under hypoxia or lactic acidosis. Second, we will use FLECS (fluorescence linked enzyme chemoproteomic strategy) to identify and optimize the selective inhibitors of the top synthetic lethal kinases. Finally, we will validate the ability of the kinase inhibitors to reduce tumor growth and metastasis. Together, we will identify and validate a set of kinase inhibitors to eradicate breast tumors in both *in vitro* culture and xenograft models of breast tumors.

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1. Introduction

Response:

We wish to clarify that COASY was identified as a kinase kit in our proposed contextual screen under lactic acidosis and hypoxia. COASY is a kinase that add the phosphate to form the final Coenzyme A product. The silencing of COASY reduce cell viability of MDA-MB231 cells. What was interesting that was that the lactic acidosis totally rescued the cell death caused by COASY silencing. In addition, COAY locus is found to be deleted in the breast cancers in TumorScape data (http://portals.broadinstitute.org/tumorscape/pages/portalHome.jsf). Therefore, we pursued the COASY as an interesting hit that can reveal how lactic acidosis select the particular somatic mutations can be selected by lactic acidosis, a prominent environmental factor in most solid tumors.

From our mechanistic studies of COASY silencing, we found that the COASY silencing to an unexpected protein hyper-acetylation due to the ability of COASY to associate and repress CBP. A previous report have shown that acidosis can reduce the histone and protein acetylation by histone deacetylases (HDACs). The released acetate anions are exported with protons out of the cell by monocarboxylate transporters (MCTs) to prevent further acidosis [1]. Therefore, we hypothesize that lactic acidosis rescued the hyperacetylation caused by COASY silencing. We will include additional data and thinking into the revised report.

The main focus of the award as outlined by the approved statement of work was to validate synthetic lethal kinases identified previously and then develop and test therapies that target the validated kinases. While the COASY data is interesting it does not fit within the scope of the SOW and is therefore not appropriate for inclusion in the first annual report. No specific information has been provided in terms of performing any experiments associated with the siRNA validation screening that was proposed.

There are comments regarding the problems associated with siRNA screening and that CRISPR-Cas is a better alternative. I am a bit confused by this argument since the SOW called for siRNA screening first followed by CRISPR-Cas validation. It is also not clear why genome wide CRISPR knock-out is necessary since the NIH award 4 R01 CA125618 10 is providing support for the general identification of synthetically lethal genes and the DoD award that you are reporting on is providing support for validation of synthetically lethal kinases which should be a rather limited subset. In the revised report only provide information relevant to this research project. In the report narrative list all tasks and sub-tasks in the SOW and after each discuss the work that was performed to complete the tasks. Do not include any results related to outside projects that are not supported by this specific award.

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We have been working on the validating the results from the synthetic lethal screens. In the revised reports, we have include some attempts to validate several lactic acidosis hits that are clustered around an amplified regions. Even with multiple efforts, the results are not yet conclusive. Therefore, we did not include these preliminary data in the original reports. COASY was reported as a kinase hit because we have extensively validated the phenotypes and made great progress on the mechanisms.

As for the CRISPR-screens, we wish to accomplish several purposes. We plan to first establish the screen methods and then focus on kinome sgRNA screens [2]. First, we are concerned whether the validation rate will be high enough to obtain a robust list of hits. Second, we hope to obtain orthogonal screens information from parallel experiments to increase the chance of success of our hits. Because of the all the concerns of the off-target effects and incomplete silencing by RNAi-based approach, we feel that it will be advantageous to update our systems of functional screens using the CRISPR-Cas9 system. With the system set up (as shown in the report), we are now focusing on validating the kinome and the kinases we have previously obtained in the previous screen results. We appreciate the feedback and suggestions from the reviewers.

1   McBrian MA, Behbahan IS, Ferrari R, Su T, Huang TW, Li K, Hong CS, Christofk HR, Vogelauer M, Seligson DB, Kurdistani SK. Histone acetylation regulates intracellular pH. Mol Cell 2013; 49: 310-321.

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As breast tumors expand in size, they rapidly exceed the local oxygen supply. A limited oxygen supply (hypoxia) forces tumor glycolysis and produces large amount of lactic acid (lactic acidosis). The regions of hypoxia and lactic acidosis contribute significantly to the treatment resistance and tumor relapse of breast cancers. While the clinical relevance of these stresses is well recognized, they are usually not incorporated in the discovery and validation phases of tumor therapeutic targets. Thus, very few options currently exist to target and eliminate cancer cells exposed to these stresses, which presents an urgent and unmet need for the breast cancer community. We hypothesize that a limited set of genes encoding protein kinases are contextually essential under hypoxia or lactic acidosis. Targeting or inhibiting these kinases will selectively eradicate tumors cells under stresses without affecting normal cells under unstressed conditions. While protein kinases are among the most chemically tractable targets, less than 10% of the human kinome has been explored for its therapeutic value. To identify these kinases with potential therapeutic values, we have performed parallel genetic (RNAi) and chemical (kinase inhibitor) "synthetic lethality" kinome screens in breast cancer cells exposed to hypoxia or lactic acidosis. Using functional genomic screens, we have identified a set of kinases which are contextually essential under hypoxia or lactic acidosis. We will first validate the inhibition of synthetic lethal kinases to eradicate breast cancer cells under hypoxia or lactic acidosis. Second, we will use FLECS (fluorescence linked enzyme chemoproteomic strategy) to identify and

optimize the selective inhibitors of the top synthetic lethal kinases. Finally, we will validate the ability of the kinase inhibitors to reduce tumor growth and metastasis. Together, we will identify and validate a set of kinase inhibitors to eradicate breast tumors in both *in vitro* culture and xenograft models of breast tumors.

Reviewer's Comments:

Thank you for submitting your first annual progress report, unfortunately the report does not meet USAMRMC standards and a revised report is required. Much of what was presented in the report pertained to investigating the impact of CoA synthase (COASY) inhibition under hypoxic and lactic acidosis conditions.

Response:

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      We wish to clarify that COASY was identified as a kinase hit in our proposed contextual screen under lactic acidosis and hypoxia. COASY is a kinase that add the phosphate to dephosphorylated CoA to form the final product Coenzyme A. The silencing of COASY reduce cell viability of MDA-MB231 cells. What was interesting that was that the lactic acidosis totally rescued the cell death caused by COASY silencing. In addition, COAY locus is found to be deleted in the breast cancers in TumorScape data (http://portals.broadinstitute.org/tumorscape/pages/portalHome.jsf). Therefore, we pursued the COASY as an interesting hit that can reveal how lactic acidosis select the particular somatic mutations can be selected by lactic acidosis, a prominent environmental factor in most solid tumors.

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We have established the individual cells with inducible Cas9. As for the CRISPR-screens, we wish to accomplish several purposes. We plan to first establish the screen methods and then focus on kinome sgRNA screens [2]. First, we are concerned whether the validation rate will be high enough to obtain a robust list of hits. Second, we hope to obtain orthogonal screens information from parallel experiments to increase the chance of success of our hits. Because of the all the concerns of the off-target effects and incomplete silencing by RNAi-based approach, we feel that it will be advantageous to update our systems of functional screens using the CRISPR-Cas9 system. With the system set up (as shown in the report), we are now focusing on the kinome and the kinases we have previously obtained in the previous screen results.

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2. Keywords: RNAi, hypoxia, lactic acidosis, functional screens, kinase, CRISPR, Cas9

3. Accomplishments

3a. The major goals of the project:

Using functional genomic screens, we have identified a set of kinases are contextually essential under hypoxia or lactic acidosis. Targeting or inhibiting these synthetic lethal kinases will selectively eradicate breast tumors cells under stresses without affecting normal cells under unstressed conditions. Therefore, we will validate the therapeutic values of these synthetic kinases and use a novel screening method to identify and optimize the kinase inhibitors and determine their ability to reduce tumor growth and metastasis.

3b. The accomplishment under these goals:

SOW:

Aim 1: Validate the inhibition of synthetic lethal kinases to eradicate breast cancer cells under hypoxia or lactic acidosis (Fiscal Years 1-2)

1) Validate the synthetic lethality using well-based siRNA under stresses -- Ongoing and partially completed

2) Validate the synthetic lethality using inducible CRISPR-based editing under stresses

Ongoing and partially completed

3) Validate the therapeutic potential of the inhibition of synthetic lethal kinases to reduce the growth and metastasis of breast cancers

Milestone 1: Obtain IACUC protocol approval --- Completed

We have made significant progress on the proposed experiments. Here, we will highlight three efforts along the proposed lines of research. First, we are reporting our efforts in the validation of several lactic acidosis synthetic lethal hits that are often amplified in the chromosomal 1q regions. In a previously published study[3], we have found that 1q is often amplified and associated with the expression of many stress-responsive genes. Therefore, we will described our efforts in the validation of these 5 hit kinase in the 1q regions.

Second, we investigate COASY, a kinase found in our screen under lactic acidosis. This gene encodes the bifunctional protein coenzyme A synthase which carries out the last two steps in the biosynthesis of CoA from pantothenic acid (vitamin B5). COASY has two domains: 1) phosphopantetheine adenylyltransferase domain of this bifunctional protein catalyzes the conversion of 4'-phosphopantetheine into dephospho-coenzyme A (dpCoA) while its 2) dephospho-CoA kinase domain completes the final step by phosphorylating dpCoA to form CoA. When COASY was silenced, the MDA-MB231 cells undergo significant cell death. Importantly, lactic acidosis totally rescued the cell death caused by COASY silencing. Third, we will discuss our efforts to establish the CRISPR-Cas9 screen in our laboratory. To validate the kinase hits

3b.1: Validation of the LA-associated hits in the 1q regions

Summary

We analyzed the list of LA synthetic lethal hits in the TCGA breast cancer data. Among the hits, we found that 5 LA synthetic lethal hits (UHMK1, SCYL3, CDK18, DSTYK and OBSCN) are co-amplified in chromosomal 1q among the similar sets of samples. Therefore, we have used different approach to silence these genes and validate their interactions with lactic acidosis in MDA-MB231 cells.

The 5 LA synthetic lethal hits (UHMK1, SCYL3, CDK18, DSTYK and OBSCN) are co-amplified among the similar sets of BRCA tumor samples (Fig 1A). Interestingly, these five kinases are physically located in the chromosomal 1q regions (Fig 1B).

In a previous study, we described the use of latent factor models to further dissect the stress-associated gene signatures in a breast cancer expression dataset[3]. We found many genes

in several latent factors are highly enriched in particular chromosomal locations. When these factors are analyzed in independent datasets with gene expression and array CGH data, the expression values of these factors are highly correlated with copy number alterations (CNAs) of the corresponding BAC clones in both the cell lines and tumors. Therefore, variation in the expression of these pathway-associated factors is at least partially caused by variation in gene dosage and CNAs among breast cancers. One previous hit is factor 26 in the chromosomal 1q regions (Fig 2A). Therefore, the expression levels of the genes in the chromosomal 1q is positively correlated with the DNA level of the corresponding regions (Fig 2B).

Therefore, we purchased shRNA and siRNA to target these kinases in the MDA-MB231 cells. We generated lentivirus to target each regions of the target kinase and transduced the cells with lentivirus. The transduced cells were then selected by puromycin to select the infected cells that have stably integrated shRNA. We then harvested the RNA of the infected cells to confirm that shRNAs have reduced the target mRNAs.

We then exposed the MDA-MB231 transduced with control or kinase-target shRNAs to control and lactic acidosis. For each target kinase, we used two independent shRNAs to avoid the off-target effects of each shRNAs. In the initial experiment using crystal violet (Fig 3, 4), we found that several shRNAs (such as CDK18 199950, DSTYK 195331) may lead to reduced cell viability under LA for ten days. Encouraged by these results, we scaled up the experiments by performing multiple replicates of the shRNA under control and LA (Fig 5). We also quantified the crystal violet using absorbance (Fig 5). Unfortunately, these experiments found that while LA reduced the crystal violet of all cells (Fig 5), none of the tested shRNAs further reduced the viability of MDA-MB231. Therefore, the expanded experiments did not validate the initial encouraging results.

We then tested different other experimental conditions and assays. First, we prolonged the period of testing from 10 to 15 days by plating fewer number of cells (Fig 6). The preliminary results indicate that longer period of exposure time may lead to stronger effects of shRNA under LA. We are expanding the experiments to include replicate. Another effort is to use Cell Titer Glow instead of crystal violet at shorter period of time (3 days). However, we did not find the interaction of any shRNAs with LA (Fig 7).

3b.2: The investigation of COASY and multinucleation phenotypes

Summary

In a forward genetic siRNA kinome screen under stresses, we found that silencing of CoA synthase (COASY) significantly reduced the cell number of MDA-MB-231 cells (Fig 8) with flattened cobble-stone appearance. Importantly, lactic acidosis totally rescued the cell death caused by COASY silencing (Fig 8). In the tumorscape data of the copy number alteration, COASY is focally deleted in breast cancers. Therefore, we feel that COASY silencing may confer survival advantages under LA.

It is important to note that COASY is included as a kinome gene of the Dharmacon kinome siRNA dataset that was used in our screen. COASY is a kinase that added the phosphate to the de-phosphorylated CoA to make CoA. Therefore, COASY is considered a kinase.

The reduced cell death caused by COASY silencing is rescued by simultaneous lactic acidosis, suggesting that this defect may offer potential advantages under lactic acidosis. CoA

synthetase (COASY) encodes a bifunctional enzyme catalyzing the last two steps of Coenzyme A (CoA) biosynthetic pathway [4]. Interestingly, we found that silencing of COASY triggered mitotic defects and multinucleation in multiple cancer cell lines. Since CoA is the major acyl carrier with a central role mediating protein acetylation, under COASY silencing, we performed acetylome analysis. This global acetylome analysis identified a generally increase in the global acetylation. Among the hits, we found COASY silencing increased the acetylation of several peptides of CREB binding protein (CBP) and targeting protein for Xenopus kinesin like protein 2 (TPX2), one crucial mitotic factor. TPX2 is a co-activator of Aurora A kinase (encoded by AURKA) that mediates AURKA localization to spindle microtubules and activates AURKA by phosphorylation [5, 6]. CBP is one of the most well-known Histone acetyltransferase which acetylate Histone H4 and activate transcription during interphase [7]. During mitosis, CBP have been reported to interact with the anaphase-promoting complex/cyclosome (APC/C) and regulate cell cycle progression by degrading mitotic proteins when cells enter anaphase [8]. Although CBP is reported to stabilize multiple non-histone proteins by acetylation [9, 10], much remains unknown about how the activities of CBP is regulated to maintain proper cell cycle progression during mitosis. During mitosis, Aurora A kinase regulates the precise timing of mitotic spindle assembly and stability through the careful regulation of its kinase activity and distinct sets of target proteins at different phase of mitosis [11]. Further experiments suggest that COASY can form a complex with CBP and inhibit the acetyltransferase activity of CBP. CBP acetylates and stabilizes TPX2 for proper cell cycle progression. CBP physically associates with acetylates TPX2 to control the Aurora A activities. CBP then acetylates and stabilizes TPX2 for proper cell cycle progression. Importantly, the CBP activities are downregulated by COASY through physical association. The depletion of COASY led to uncontrolled CBP activity during mitosis and an exaggerated level/acetylation of TPX2, persistent TPX2 and prolonged Aurora A activation, which contributes to the mitotic defects and multi-nucleation phenotypes. Since timely regulated TPX2 level ensures the proper progression of mitosis [12, 13], uncontrolled level of TPX2 can lead mitotic defect and multinucleation. Our findings reveal a novel mechanism by which a metabolic enzyme, COASY, regulates the progression of mitosis by controlling the timing and activities of acetyltransferase activity of CBP.

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COASY silencing trigger multinucleation phenotypes

In a forward genetic siRNA screen, we found that silencing of CoA synthase (COASY) significantly reduced the cell number of MDA-MB-231 cells rescued by lactic acidosis (Fig 8). COASY silencing renders cells flattened cobble-stone appearance (Fig 9A, B). By staining with DAPI and F-actin, we found that COASY silencing triggered prominent multi-nucleation phenotypes in multiple cells (Fig 9D-1F). COASY encodes CoA synthase that is responsible for the last two steps of *de novo* CoA synthesis from pantothenic acid (vitamin B5). Therefore, we determined how COASY silencing impacted the level of CoA and acetyl-CoA using LC-MS analysis. We found that COASY silencing reduced both CoA and acetyl-CoA by ~50%, indicating a successful silencing and inhibition of the CoA and acetyl-CoA synthesis (data not shown). These reduced CoA and acetyl-CoA indicated that COASY has been successfully silenced.

Next, we determined whether enzymatic activities are required for the COASY-mediated multinucleation phenotypes. The R499C mutation of COASY in human is known to disrupt its enzymatic activity and associate with the development of neurodegeneration with iron accumulation [14]. To test the role of enzymatic activities of COASY in the multi-nucleation phenotypes, we transfect wild type and enzymatic dead mutant (R499C) of COASY into the COASY silenced cells to determine their ability for rescue the aneuploidy phenotypes caused by

COASY silencing. We found that both wild type and enzymatic dead mutant of COASY could completely rescue the multinucleation phenotypes (Fig 9E).

COASY encode the last protein in the multi-step steps during the de no synthesis of CoA from panthenate acid (Vitamin B5). Mutations in PANK2 and CoASY lead to PKAN and CoPAN forms of NBIA, respectively. To further determine the role of CoA synthesis in the multi-nucleation phenotypes, we inhibit other proteins in the pathways using Hopan (PANK inhibitors) as well as siRNAs against PANK and PPCDC. None of these inhibitors or siRNAs induced the multi-nucleation phenotypes seen with COASY silencing (Fig 9F). Collectively, these results indicate that COASY regulates the CBP activities and mitotic fidelity via an enzymatic independent manner.

COASY silencing increased TPX2 induction and lysine-acetylation

Given the importance of CoA and acetyl-CoA as substrates in the reversible lysine acetylation (K-Ac or Ac-K), we perform a global acetylome analysis using mass spectrometry (MS) to determine the how COASY silencing affects the lysine acetylation (K-Ac) of cells during mitosis. Such global acetylome analysis indicated a general hyper-acetylation of a large number of proteins that include histones and histone acetyl transferase CREB Binding Protein (CBP) (Fig 10A,B). The increased lysine acetylation in CBP suggests increased enzymatic activities [25], consistent with increased acetylation upon COASY silencing. In addition, mass spec analysis identified a consistent 5-10 fold increase in three acetylated peptides of TPX2 (K75, K476 and K582) (Fig 10C). TPX2 is a co-activator of Aurora A kinase (encoded by AURKA) that mediates AURKA localization to spindle microtubules and activates AURKA by phosphorylation [5, 6]. During mitosis, Aurora A kinase regulates the precise timing of mitotic spindle assembly and stability through the careful regulation of its kinase activity and distinct sets of target proteins at different phase of mitosis [11]. One important regulatory mechanism is the co-activator TPX2, which allosterically regulates Aurora-A kinase activity through the autophosphorylation of Aurora A.

The increased levels of multiple acetylated TPX2 peptides suggest an increase in the overall protein levels and lysine acetylation of TPX2 that is expected to activate Aurora A kinase. As expected, immunoblots revealed that COASY silencing increased both TPX2 protein levels as well as Aurora A phosphorylation (Thr288) (Fig 10D). This increased Aurora A phosphorylation caused by COASY silencing can be abolished by simultaneous transfection of TPX2 siRNA (Fig 10D). This result suggested that COASY silencing triggered Aurora A phosphorylation by increasing TPX2 expression.

The stage-specific interaction between COASY, TPX2 and CBP during cell cycle progression

We hypothesize that COASY may regulate the TPX2 acetylation by CBP through physical interaction. To test the potential interaction between COASY, TPX2 and CBP during mitosis, we used confocal microscopy to determine the physical locations of TPX2 and CBP in COASY-GFP expressing cells. While the physical location of COASY did not significantly overlap with TPX2 and CBP during interphase (Fig 11A-B), most COASY-GFP are found to co-localize with CBP during mitosis (Fig 11A). Furthermore, significant portions of COASY-GFP are found at centrosome that overlap with TPX2 during early mitosis (Fig. 11B). This data suggests that COASY, TPX2 and CBP might interact with each other during early mitosis. To further confirm physical interaction between COASY, TPX2 and CBP during early mitosis, we treated cells with thymidine-nocodazole block to arrest the cell population in early mitosis. After a mitotic shake-off to enrich the mitotic cell population, cells were then released in fresh media at different time points. The endogenous CBP was immunoprecipitated using CBP antibody and associated proteins were then analyzed by immunoblotting (Fig. 11C). Both COASY and TPX2

showed robust interaction with CBP between 20 to 40 min after releasing from nocodaozle during the transition from early to late mitosis. This association disappeared after 70 min post-release. Thus, COASY, TPX2 and CBP are physically associated during early mitosis.

To understand the functional relevance of COASY during the mitotic progression, we determined how COASY silencing affects cell cycle progression, similar to Fig 11C. First, we found that COASY silencing significantly prolonged mitosis as cyclin B1, expressed predominantly during G2/M phase, was found over extended periods of time (Fig 11D). COASY silencing also delayed its downregulation from 40 min (siControl) to 140 min (siCOASY) after release (Fig 11D). Additionally, Western blotting of CBP under control siRNA showed two predominant bands. While the lower band of CBP was ubiquitously expressed, the upper band of CBP was cell cycle-regulated and highly expressed during early mitosis. COASY silencing increased the portion of the upper band of CBP over an extended periods of mitosis. When the protein extracts were treated with Lambda protein phosphatase, the higher molecular weight bands of CBP disappeared and were replaced with the CBP of lower molecular band (Fig 11E). These results suggest the higher molecular weight bands of CBP were caused by phosphorylation, consistent with previous reports on the cell-cycle specific CBP phosphorylation [15] that is associated with enhanced HAT activities [15]. To determine the ability of COASY to regulate CBP, we pulled down CBP by either antibody against CBP or COASY and found that COASY mostly associated with the un-phosphorylated CBP (Fig 11F). In contrast, CBP antibody pull down both phosphorylated and unphosphorylated CBP. Furthermore, when normalized against the similar amount of CBP, COASY-associated CBP has a dramatically reduced histone acetyl-transferase activities (Fig 11G). Therefore, these data suggest that COASY may bind to CBP and inhibit its HAT activities.

The acetylation on three lysine residues of TPX2 is crucial for TPX2 stability and Aurora A phosphorylation

Since COASY silencing increased TPX2 and CBP acetylation (Fig 10B-C) and COASY-CBP-TPX2 physical interaction during early mitosis (Fig 11B), we speculated that COASY may regulate the CBP-mediated TPX2 acetylation. We first verified the increased TPX2 acetylation identified by MS (Fig 10D). We pulled down TPX2 and probed with lysine acetylation antibodies. These experiments reveal that COASY silencing increased TPX2 acetylation (Fig 12A). To determine whether TPX2 is a target protein of CBP, we co-transfected wildtype (CBP WT) and enzymatic dead mutant (CBP Mut) CBP with TPX2 and test TPX2 acetylation (Fig 12B). After 24 hours of transfection, the early mitosis cells were enriched and TPX2 was immunoprecipitated and analyzed by immunoblotting (Fig 12B). These data showed wildtype CBP, but not vector or enzymatic dead mutants, increased the lysine acetylation and expression of TPX2.These data suggests that CBP acetylates TPX2 and regulates the expression level of TPX2by its enzymatic activities.

Next, we determined the role of three lysine residues of TPX2 (K75, K476 and K582) whose acetylation was increased by COASY silencing. We made acetylation-deficient mutants by converting lysine to arginine (TPX2-RRR at K75R, K476R and K582R). We co-transfected CBP expression constructs with either wildtype or triple mutant form of TPX2 (TPX2-RRR) to 293T cells (Fig 12B). When the pulled down TPX2 were probed with acetylated lysine antibody, TPX2 triple mutant showed ~50% reduction in lysine acetylation, suggesting that these three lysine residues play an important role in the CBP–mediated TPX2 acetylation. Furthermore, CBP co-transfection stabilized TPX2 and increased its expression by ~ 3 fold (Fig 12B). However, this CBP-mediated stabilization was not found for TPX2 triple mutant (TPX2-RRR). Together, these results suggest the important role of these three acetylated lysine residues in regulating the TPX2 protein level by CBP.

To determine to the role of these lysine residues for Aurora A phosphorylation, we cotransfected Aurora A expression constructs with wildtype or triple mutant form of TPX2 (TPX2-RRR) in 293T cells. Similar to Fig 4B, TPX2 triple mutant, when compared with wild type, showed reduced ability to increase Aurora A phosphorylation (Fig 12C). These results suggest that these three TPX2 lysine residues not only regulate the stability of TPX2 but also critical in activating Aurora A.

CBP inactivation and TPX2 silencing rescues COASY dependent multinucleation

To investigate the importance of CBP in the COASY-silenced phenotypes, we used CBP inhibitor C646 and found C646 reduced both TPX2 upregulation and increased Aurora A phosphorylation associated with COASY silencing (Fig 13A). In addition, the inhibition of CBP by siRNA and C646 abolished the multinucleation induced by COASY silencing (Fig 13B-C). Next, we determined the role of TPX2 in the COASY silencing phenotypes. During the COASY silencing, we also depleted TPX2 by different amount of TPX2 siRNAs, we found that the TPX2 siRNAs rescued the COASY-silencing multinucleation phenotypes in a dose-dependent manner (Fig 13D). Therefore, the increased CBP and TPX2 caused by COASY silencing is essential for the multi-nucleation phenotypes.

Based on these data, we propose a model (Fig 13D) in which that CBP play an important role in the cell-cycle-specific activation of Aurora-A by the TPX2 acetylation at three lysine residues via direct interaction between CBP and TPX2. The COASY is important for the inactivation of CBP to turn off the TPX2 and Aurora A, which is required for cell cycle progression. The regulation of the TPX2 is mediated by a cell-cycle-specific association of COASY with CBP. The silencing of COASY leas to the hyper-acetylation of TPX2 and activation of Aurora A kinase. Such increased Aurora A kinase contributes to the mitotic defects and multi-nucleation phenotypes. These data revealed an unexpected role of tumor metabolisms in regulating the mitotic fidelity and proper cell cycle progression.

Taken together, in addition to the involvement of de novo CoA biosynthesis pathway, our finding identified a non-canonical role of COASY in regulating the CBP localization and acetyltransferase activity during mitosis, which in term stabilize and activate TPX2 for the proper progression of mitosis. Importantly, during mitosis, the nuclear envelope breakdown and chromosome condensation render transcription inactive [16-19]. However, our results indicate an increase in the level of TPX2 under COASY silencing during mitosis, which point out the importance of post-transcriptional modification by CBP in regulating the level of TPX2 and potential more mitotic proteins.

From our mechanistic studies of COASY silencing, we found that the COASY silencing to an unexpected protein hyper-acetylation due to the ability of COASY to associate and repress CBP. A previous report have shown that acidosis can reduce the histone and protein acetylation by histone deacetylases (HDACs). The released acetate anions are exported with protons out of the cell by monocarboxylate transporters (MCTs) to prevent further acidosis [1]. Therefore, we hypothesize that lactic acidosis rescued the hyperacetylation caused by COASY silencing to rescue the COASY-regulated CBP-mediated TPX2 acetylation and Aurora A activation.

3b.3 Setting up CRISPR-Cas9 system for single gene validation and screens

For loss-of-function studies in mammalian cells, use of RNA interference (RNAi) is currently the standard approach for its rapid and easy implementation. Genome-scale RNAi screens have been adopted as a standard approach by cancer researchers and have demonstrated utility in discovering novel cancer genes, synthetic lethal interactions with oncogenes, drug resistance mechanisms and potential therapeutic targets [20-23]. Therefore, our initially proposed

functional kinome screens were performed using RNAi based by shRNA and siRNA-mediated gene silencing. However, with maturation of the RNAi technology and the bioinformatics methodology to analyze RNAi experiments, we have begun to better appreciate some of the limitations. The RNAi-based approaches suffer from potential off-target effects and partial gene suppression. We also feel that these off-target effects may be responsible for many false-positive hits in our original screen as well as why different shRNAs have different effects.

These limitations can be problematic in high-throughput applications, where there have been several reports of off-target effects on a few key genes largely determining screen hits [24-26]. Such off-target effects necessitate thorough validation and characterization of candidates arising from RNAi screens to establish them as bona fide hits. Additionally, the fact that many RNAi sequences elicit only partial suppression of their targets effectively prevents screens from reaching saturation, leading to high rates of false-negatives and lack of concordance between screens for the same phenotype [27]. Together, these caveats constrain the utility of RNAi libraries, but in the absence of any other alternatives for systematic loss-of-function studies, they were the best tools available until recently.

CRISPR-Cas9 gene editing/deletion

In early 2013, several groups reported the discovery that the prokaryotic immune defense system consisting of clustered regularly interspaced short palindromic repeats (CRISPR) and Cas9 endonucleases can be repurposed for the rapid and simple editing of genomes of many organisms, including humans [28-30]. In this approach, concomitant introduction of Cas9 with a guide RNA (gRNA) complementary to the targeted gene leads to double-stranded breaks in the genome, which are then repaired by error-prone non-homologous end joining, frequently leading to insertions or deletions that cause gene-inactivating frame-shifts (Figure1). In December 2013, several groups reported genome-scale libraries of CRISPR gRNAs for high-throughput targeting of human or mouse genes, demonstrating their utility for functional screening applications [2, 31, 32]. While this technology is very new, early work suggests that the efficiency of CRISPR-mediated loss-of-function is higher and the prevalence of off-target effects is lower than current RNAi-based methods.

The development of CRISPR-Cas9 based technology has radically altered the landscape of molecular biology by enabling easy and rapid genome editing in virtually any cell type or organism [28-30]. Cas9 can be programmed with a single guide RNA (sgRNA) to target specific genomic sequences, where it introduces double-strand breaks (DSBs) (Fig 14). The broad utility and accessibility of CRISPR-mediated genome editing is made evident by the widespread adoption of CRISPR-based techniques by many labs to generate knockout or knock-in experimental models in the short two years since its introduction. Of particular interest to our Facility, CRISPR technologies have also enabled the development of new genome-scale libraries that can be used to interrogate gene function in systematic, high-throughput fashion [2, 32, 33]. Such libraries have demonstrated robust and reproducible performance in identifying genes and pathways involved in such diverse processes as cancer cell proliferation, innate immune response, virus-induced cell death, tumor metastasis and cancer drug resistance [2, 32, 34-38]. Moreover, CRISPR-based libraries outperform the previously available shRNA libraries by a wide margin, with better consistency of reagents against the same gene and higher validation rates. This is likely attributable at least in part to the complete loss of function phenotypes and fewer off-target effects associated with CRISPR approaches [32, 35, 39].

Derive a clonal cell line expressing FLAG-Cas9 under the control of a doxycycline responsive promoter (pCW-Cas9)

To facility the screen and functionally validation our kinase hits, we generate multiple single cell clones with low background and highly inducible Cas9 in MDA-MB231 cells. We used the inducible system developed by Wang et al [2]. First, we used the pCW-Ca9 to generate Cas9 lentivirus. Next, we transduce target cells (MDA-MB231) followed by puromycin selection 24 hours after infection. Then we isolate single-cell clones via limiting dilution. We tested the individual single cell clones for their baseline and inducible expression of for Cas9 expression via western blotting using an anti-FLAG antibody. The purpose was to select for the single cell clones which have no expression of Cas9 before tetracycline (before induction) as well as the highest induction of Cas9 expression after Dox induction. As shown in Figure 8, we have generated up 10 ten individual MDA-MB231 with inducible Cas9 expression and appropriate for screening after the additional transfection of the sgRNA library (Fig 15).

Pilot in vitro CRISPR screens for functional dependencies in cancer cells

In our first year, we sought to generate reagents, develop experimental protocols and establish analysis pipelines for the use of CRISPR sgRNA libraries targeting the human genome in order to evaluate the feasibility and performance of the technology for high-throughput loss-of-function screening. To this end, we performed pilot screens using a cell line for which we have genome mutation data, facilitating prioritization of genetic dependencies that may be targeted for therapeutic intervention. We tested two different pooled lentiviral sgRNA libraries for CRISPR-Cas9-based loss-of-function screening, targeting 7,117 and 18,080 human genes with 72,151 and 123,411 unique sgRNAs respectively [2, 32]. The smaller library uses Tet-regulated expression of the Cas9 nuclease for inducible gene editing, while the larger library, also known as GeCKO (Genome-scale CRISPR Knock-Out), uses constitutively expressed CAS9. After amplification of library DNA stocks (Fig 16) , we were able to verify >95% retention of all library sgRNAs (Fig 8), as assessed by deep sequencing. Both libraries were then used to screen cancer cell line to identify genes that regulate cell proliferation, using protocols modified from those previously designed for pooled screening with lentiviral shRNA libraries. We also tested and optimized procedures for sample processing, deep sequencing for screen deconvolution and data analysis.

Our work demonstrated robust performance of the CRISPR screening technology (Fig 17-18), as evidenced by our ability to identify known essential genes and pathways using both libraries. We identified processes well known to be generally required for proliferation, including translation, transcription, splicing, DNA replication and proteasome function. We also recovered several expected genes, such as MYC and BCL6, which are known to be mutated in many cancers. We also identified several novel genes where knockout appeared to promote cell proliferation, suggesting possible tumor suppressor function, including TOX. In parallel with these experiments, we also investigated the ability of both library vector systems to induce mutations at individual loci and found mutation rates of 80-100% in most cell lines and loci tested, suggesting that lentiviral delivery enables highly efficient genome editing. This is due at least in part to the stable expression of Cas9 and sgRNAs enabled by lentiviral transduction, leading to successive cycles of DSB formation and repair until the genome is mutated, preventing further recognition by the sgRNA/Cas9 complex. As the GeCKO library has broader coverage of the human genome and slightly better performance metrics both at individual loci and genome wide, we anticipate that most screens will be performed using the GeCKO system. Our results established CRISPR sgRNA genome-wide loss-of-function pooled screening in human cancer cell lines as a viable method for DCI investigators.
We have begun perform synthetic lethal kinome screens of MDA-MB231 under hypoxia and lactic acidosis using cells with inducible Cas9 by doxycycline.

3c. Opportunities for training and professional development has the project provided?

The proposed projects have supported ample opportunities for training and professional developments. The project is currently supporting two PhD students enrolled in the University Program in Genetics and Genomics (UPGG). The funded research afford them the projects and the chances for their graduate training and professional development. They have taken training courses for bioinformatics as well as mass spectrometry analysis to further their graduate training for their future career in science.

3d. How were the results disseminated to communities of interest?

The results are disseminated to communities by multiple ways. First, we have been publishing our results in several publications to dissect our results to the scientific communities [40-43]. The original profiling data from the screens and gene expression were also deposited into the GEO database to make the data available for the scientific community. In addition, both the PIs and trainees have presented these findings in AACR and Gordon conferences to share the results with other investigators in the related fields.

3e. What do you plan to do during the next reporting period to accomplish the goals?

We will continue to validate the screen hits of the genetic determinants of the cellular response to hypoxia and acidosis. We will continue to validate these hits and investigate the mechanisms by which these genes and proteins affect the cellular survival under hypoxia and lactic acidosis.

4. Impact

4a. What was the impact on the development of the principal discipline(s) of the project?

We have published 13 peer review articles and one edited book. These papers and their data have been released to the scientific community.

4b. **What was the impact on other disciplines?**

Nothing to Report

4c. What was the impact on technology transfer?

Nothing to Report

4d. What was the impact on society beyond science and technology?

Nothing to Report

5. Changes/Problems

Nothing to Report

6. Products.

Peer Review Journal Articles

1. Lewis D.A., Suchindran S., Beckman M.G., Hooper W.C., Grant A.M., Heit J.A., Manco-Johnson M., Moll S., Philipp C.S., Kenney K., De Staercke C., Pyle M.E., **Chi J.T.**, Ortel T.L. (2015) "Whole blood gene expression profiles distinguish clinical phenotypes of venous thromboembolism". *Thrombosis Research*. 2015 Apr;135(4):659-65. PMCID: PMC4493863

2. Mo L., Bachelder R.E., Kennedy M., Chen P.H., **Chi J.T.**, Berchuck A., Cianciolo G., Pizzo S.V. (2015) "Syngeneic Murine Ovarian Cancer Model Reveals That Ascites Enriches for Ovarian Cancer Stem-Like Cells Expressing Membrane GRP78" *Molecular Cancer Therapeutics* 2015 Mar;14(3):747-56. PMCID: In Progress

3. Tang X., Keenan M.M., Wu J., 2, Lin C.A., Dubois L, Thompson J.W., Freedland S.J., Murphy S.K., and **Chi J.T.** (2015) "Comprehensive profiling of amino acid response uncovers unique methionine-deprived response dependent on intact creatine biosynthesis" *PLoS Genetics* Apr 7;11(4):e1005158. PMCID: PMC4388453

4. Keenan M.M., **Chi J.T.** (2015) "Alternative fuels for cancer cells". *Cancer J.* 2015 Mar-Apr;21(2):49-55. PMCID: PMC4380238

5. Jiang X., Nevins J.R., Shats I., **Chi J.T.** (2015) "E2F1-Mediated Induction of NFYB Attenuates Apoptosis via Joint Regulation of a Pro-Survival Transcriptional Program" *PLoS One*. 2015 Jun 3;10(6):e0127951.

6. Kephart J.J., Tiller R.G., Crose L., Slemmons K.K., Chen P.H., Hinson A.R., Bentley R.C., **Chi J.T.**, Linardic C.M. (2015) "Secreted frizzled related protein 3 (SFRP3) is required for tumorigenesis of PAX3-FOXO1-positive alveolar rhabdomyosarcoma" *Clin Cancer Res*. 2015 Jun 12

7. Mercer J.L., Argus J.P., Crabtree D.M., Keenan M.M., Wilks M.Q., **Chi J.T.**, Bensinger S.J., Lavau C.P., Wechsler D.S. (2015) "Modulation of PICALM Levels Perturbs Cellular Cholesterol Homeostasis" *PLoS One*. 2015 Jun 15; 10 (6):e0129776. PMCID: PMC4467867

8. Horton J.K., Blitzblau R.C., Yoo S., Geradts J., Chang Z., Baker J.A., Georgiade G.S., Chen W., Siamakpour-Reihani S., Wang C., Broadwater G., Groth J., Palta M., Dewhirst M., Barry W.T., Duffy E.A., **Chi J.T.**, Hwang E.S. (2015) "Preoperative Single-Fraction Partial Breast Radiation Therapy: A Novel Phase 1, Dose-Escalation Protocol With Radiation Response Biomarkers"
Int J Radiat Oncol Biol Phys. 2015 Jul 15;92 (4):846-55. PMCID: PMC4481883

9. LaGory E.L., Wu C., Taniguchi C.M., Ding C.K., **Chi J.T.**, von Eyben R., Scott D.A., Richardson A.D., Giaccia A.J. "Suppression of PGC-1α Is Critical for Reprogramming

Oxidative Metabolism in Renal Cell Carcinoma" *Cell Rep.* 2015 Jul 7;12(1):116-27. PMCID: PMC4518559

10. Bhattacharyya J., Bellucci J.J., Weitzhandler I., McDaniel J.R., Spasojevic I., Li X., Lin C.C., **Chi J.T.**, Chilkoti A. (2015). "A paclitaxel-loaded recombinant polypeptide nanoparticle outperforms Abraxane in multiple murine cancer models" *Nat Commun.* 2015 Aug 4;6:7939. PMCID: In Progress

11. Kwon D.Y., Lee H.E., Weitzel D.H., Park K., Lee S.H., Lee C.T., Stephenson T.N., Park H., Fitzgerald M.C., **Chi J.T.**, Mook R.A. Jr, Dewhirst M.W., Lee Y.M., Hong J. "Synthesis and Biological Evaluation of Manassantin Analogues for Hypoxia-Inducible Factor 1α Inhibition" *J Med Chem.* 2015 Oct 8;58(19):7659-71. PMCID: In Progress

12. Keenan M.M., Liu B., Tang X., Wu J., Cyr D., Stevens R.D., Ilkayeva O., Huang Z., Tollini L.A., Murphy S.K., Lucas J., Muoio D.M., Kim S., **Chi J.T.** (2015) "ACLY and ACC1 Regulate Hypoxia-Induced Apoptosis by Modulating ETV4 via α-ketoglutarate" *PLoS Genetics* 2015 Oct 9;11(10). PMCID: In Progress

13. Horton J,K., Siamakpour-Reihani S., Lee C.T., Zhou Y., Chen W., Geradts J., Fels D.R., Hoang P., Ashcraft K.A., Groth J., Kung H.N., Dewhirst M.W., and **Chi J.T.** (2015) "FAS death receptor: a breast cancer subtype-specific radiation response biomarker and potential therapeutic target" *Radiation Research*, In Press. PMCID: In Progress

Editor of one book:

Chi J.T. (Ed.). (2015) "Molecular Genetics of Dysregulated pH Homeostasis" New York, NY: Springer Press. In press.

7. Participants & Other Collaborating Organizations

No change

8. Special Reporting Requirements

Not applicable

9. Appendices

Figures for the Progress Report.

10. References:

1.	McBrian MA, Behbahan IS, Ferrari R, Su T, Huang TW, Li K, Hong CS, Christofk HR, Vogelauer M, Seligson DB *et al*: **Histone acetylation regulates intracellular pH**. *Mol Cell* 2013, **49**(2):310-321.

2.	Wang T, Wei JJ, Sabatini DM, Lander ES: **Genetic screens in human cells using the CRISPR-Cas9 system**. *Science* 2014, **343**(6166):80-84.

3.	Lucas JE, Kung HN, Chi JT: **Latent factor analysis to discover pathway-associated putative segmental aneuploidies in human cancers**. *PLoS Comput Biol* 2010, **6**(9):e1000920.

4.	Zhyvoloup A, Nemazanyy I, Babich A, Panasyuk G, Pobigailo N, Vudmaska M, Naidenov V, Kukharenko O, Palchevskii S, Savinska L *et al*: **Molecular cloning of CoA Synthase. The missing link in CoA biosynthesis**. *J Biol Chem* 2002, **277**(25):22107-22110.

5.	Kufer TA, Silljé HHW, Körner R, Gruss OJ, Meraldi P, Nigg EA: **Human TPX2 is required for targeting Aurora-A kinase to the spindle**. *The Journal of Cell Biology* 2002, **158**(4):617-623.

6.	Eyers PA, Maller JL: **Regulation of Xenopus Aurora A activation by TPX2**. *J Biol Chem* 2004, **279**(10):9008-9015.

7.	Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y: **The transcriptional coactivators p300 and CBP are histone acetyltransferases**. *Cell* 1996, **87**(5):953-959.

8.	Turnell AS, Stewart GS, Grand RJ, Rookes SM, Martin A, Yamano H, Elledge SJ, Gallimore PH: **The APC/C and CBP/p300 cooperate to regulate transcription and cell-cycle progression**. *Nature* 2005, **438**(7068):690-695.

9.	van der Heide LP, Smidt MP: **Regulation of FoxO activity by CBP/p300-mediated acetylation**. *Trends Biochem Sci* 2005, **30**(2):81-86.

10.	Ito A, Lai CH, Zhao X, Saito S, Hamilton MH, Appella E, Yao TP: **p300/CBP-mediated p53 acetylation is commonly induced by p53-activating agents and inhibited by MDM2**. *EMBO J* 2001, **20**(6):1331-1340.

11.	Fu J, Bian M, Jiang Q, Zhang C: **Roles of Aurora kinases in mitosis and tumorigenesis**. *Mol Cancer Res* 2007, **5**(1):1-10.

12.	Kufer TA, Sillje HH, Korner R, Gruss OJ, Meraldi P, Nigg EA: **Human TPX2 is required for targeting Aurora-A kinase to the spindle**. *J Cell Biol* 2002, **158**(4):617-623.

13.	Wittmann T, Wilm M, Karsenti E, Vernos I: **TPX2, A novel xenopus MAP involved in spindle pole organization**. *J Cell Biol* 2000, **149**(7):1405-1418.

14.	Dusi S, Valletta L, Haack TB, Tsuchiya Y, Venco P, Pasqualato S, Goffrini P, Tigano M, Demchenko N, Wieland T *et al*: **Exome sequence reveals mutations in CoA synthase as a cause of neurodegeneration with brain iron accumulation**. *Am J Hum Genet* 2014, **94**(1):11-22.

15.	Ait-Si-Ali S, Ramirez S, Barre FX, Dkhissi F, Magnaghi-Jaulin L, Girault JA, Robin P, Knibiehler M, Pritchard LL, Ducommun B *et al*: **Histone acetyltransferase activity of**

CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature* 1998, **396**(6707):184-186.

16. Prescott DM, Bender MA: **Synthesis of RNA and protein during mitosis in mammalian tissue culture cells**. *Exp Cell Res* 1962, **26**:260-268.

17. Taylor JH: **Nucleic acid synthesis in relation to the cell division cycle**. *Ann N Y Acad Sci* 1960, **90**:409-421.

18. Johnson TC, Holland JJ: **Ribonucleic acid and protein synthesis in mitotic HeLa cells**. *J Cell Biol* 1965, **27**(3):565-574.

19. Gottesfeld JM, Forbes DJ: **Mitotic repression of the transcriptional machinery**. *Trends Biochem Sci* 1997, **22**(6):197-202.

20. Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, Schinzel AC, Sandy P, Meylan E, Scholl C *et al*: **Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1**. *Nature* 2009, **462**(7269):108-112.

21. Hagerstrand D, Tong A, Schumacher SE, Ilic N, Shen RR, Cheung HW, Vazquez F, Shrestha Y, Kim SY, Giacomelli AO *et al*: **Systematic interrogation of 3q26 identifies TLOC1 and SKIL as cancer drivers**. *Cancer discovery* 2013, **3**(9):1044-1057.

22. Prahallad A, Sun C, Huang S, Di Nicolantonio F, Salazar R, Zecchin D, Beijersbergen RL, Bardelli A, Bernards R: **Unresponsiveness of colon cancer to BRAF(V600E) inhibition through feedback activation of EGFR**. *Nature* 2012, **483**(7387):100-103.

23. Bivona TG, Hieronymus H, Parker J, Chang K, Taron M, Rosell R, Moonsamy P, Dahlman K, Miller VA, Costa C *et al*: **FAS and NF-kappaB signalling modulate dependence of lung cancers on mutant EGFR**. *Nature* 2011, **471**(7339):523-526.

24. Adamson B, Smogorzewska A, Sigoillot FD, King RW, Elledge SJ: **A genome-wide homologous recombination screen identifies the RNA-binding protein RBMX as a component of the DNA-damage response**. *Nature cell biology* 2012, **14**(3):318-328.

25. Ma Y, Creanga A, Lum L, Beachy PA: **Prevalence of off-target effects in Drosophila RNA interference screens**. *Nature* 2006, **443**(7109):359-363.

26. Sigoillot FD, Lyman S, Huckins JF, Adamson B, Chung E, Quattrochi B, King RW: **A bioinformatics method identifies prominent off-targeted transcripts in RNAi screens**. *Nature methods* 2012, **9**(4):363-366.

27. Bushman FD, Malani N, Fernandes J, D'Orso I, Cagney G, Diamond TL, Zhou H, Hazuda DJ, Espeseth AS, Konig R *et al*: **Host cell factors in HIV replication: meta-analysis of genome-wide studies**. *PLoS pathogens* 2009, **5**(5):e1000437.

28. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Marraffini LA *et al*: **Multiplex genome engineering using CRISPR/Cas systems**. *Science* 2013, **339**(6121):819-823.

29. Jinek M, East A, Cheng A, Lin S, Ma E, Doudna J: **RNA-programmed genome editing in human cells**. *eLife* 2013, **2**:e00471.

30. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM: **RNA-guided human genome engineering via Cas9**. *Science* 2013, **339**(6121):823-826.

31. Koike-Yusa H, Li Y, Tan EP, Velasco-Herrera MD, Yusa K: **Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library**. *Nature biotechnology* 2013.

32. Shalem O, Sanjana NE, Hartenian E, Shi X, Scott DA, Mikkelsen TS, Heckl D, Ebert BL, Root DE, Doench JG *et al*: **Genome-scale CRISPR-Cas9 knockout screening in human cells**. *Science* 2014, **343**(6166):84-87.

33. Koike-Yusa H, Li Y, Tan EP, Velasco-Herrera Mdel C, Yusa K: **Genome-wide recessive genetic screening in mammalian cells with a lentiviral CRISPR-guide RNA library**. *Nature biotechnology* 2014, **32**(3):267-273.

34. Shi J, Wang E, Milazzo JP, Wang Z, Kinney JB, Vakoc CR: **Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains**. *Nature biotechnology* 2015, **33**(6):661-667.

35. Shalem O, Sanjana NE, Zhang F: **High-throughput functional genomics using CRISPR-Cas9**. *Nature reviews Genetics* 2015, **16**(5):299-311.

36. Parnas O, Jovanovic M, Eisenhaure TM, Herbst RH, Dixit A, Ye CJ, Przybylski D, Platt RJ, Tirosh I, Sanjana NE *et al*: **A Genome-wide CRISPR Screen in Primary Immune Cells to Dissect Regulatory Networks**. *Cell* 2015, **162**(3):675-686.

37. Ma H, Dang Y, Wu Y, Jia G, Anaya E, Zhang J, Abraham S, Choi JG, Shi G, Qi L *et al*: **A CRISPR-Based Screen Identifies Genes Essential for West-Nile-Virus-Induced Cell Death**. *Cell reports* 2015, **12**(4):673-683.

38. Chen S, Sanjana NE, Zheng K, Shalem O, Lee K, Shi X, Scott DA, Song J, Pan JQ, Weissleder R *et al*: **Genome-wide CRISPR screen in a mouse model of tumor growth and metastasis**. *Cell* 2015, **160**(6):1246-1260.

39. Hartenian E, Doench JG: **Genetic screens and functional genomics using CRISPR/Cas9 technology**. *The FEBS journal* 2015, **282**(8):1383-1393.

40. Tang X, Wu J, Ding C-K, Lu M, Keenan MM, Lin C-C, Lin C-A, Wang CC, George D, Hsu DS *et al*: **Cystine deprivation triggers programmed necrosis in VHL-deficient renal cell carcinomas**. *Cancer Research* 2016.

41. Keenan MM, Liu B, Tang X, Wu J, Cyr D, Stevens RD, Ilkayeva O, Huang Z, Tollini LA, Murphy SK *et al*: **ACLY and ACC1 Regulate Hypoxia-Induced Apoptosis by Modulating ETV4 via alpha-ketoglutarate**. *PLoS Genet* 2015, **11**(10):e1005599.

42. Tang X, Keenan MM, Wu J, Lin CA, Dubois L, Thompson JW, Freedland SJ, Murphy SK, Chi JT: **Comprehensive profiling of amino acid response uncovers unique methionine-deprived response dependent on intact creatine biosynthesis**. *PLoS Genet* 2015, **11**(4):e1005158.

43. Tang X, Lin CC, Spasojevic I, Iversen E, Chi JT, Marks JR: **A joint analysis of metabolomics and genetics of breast cancer** *Breast Cancer Res* 2014, **16**(4):415.

Synthetic Lethal Hits in LA
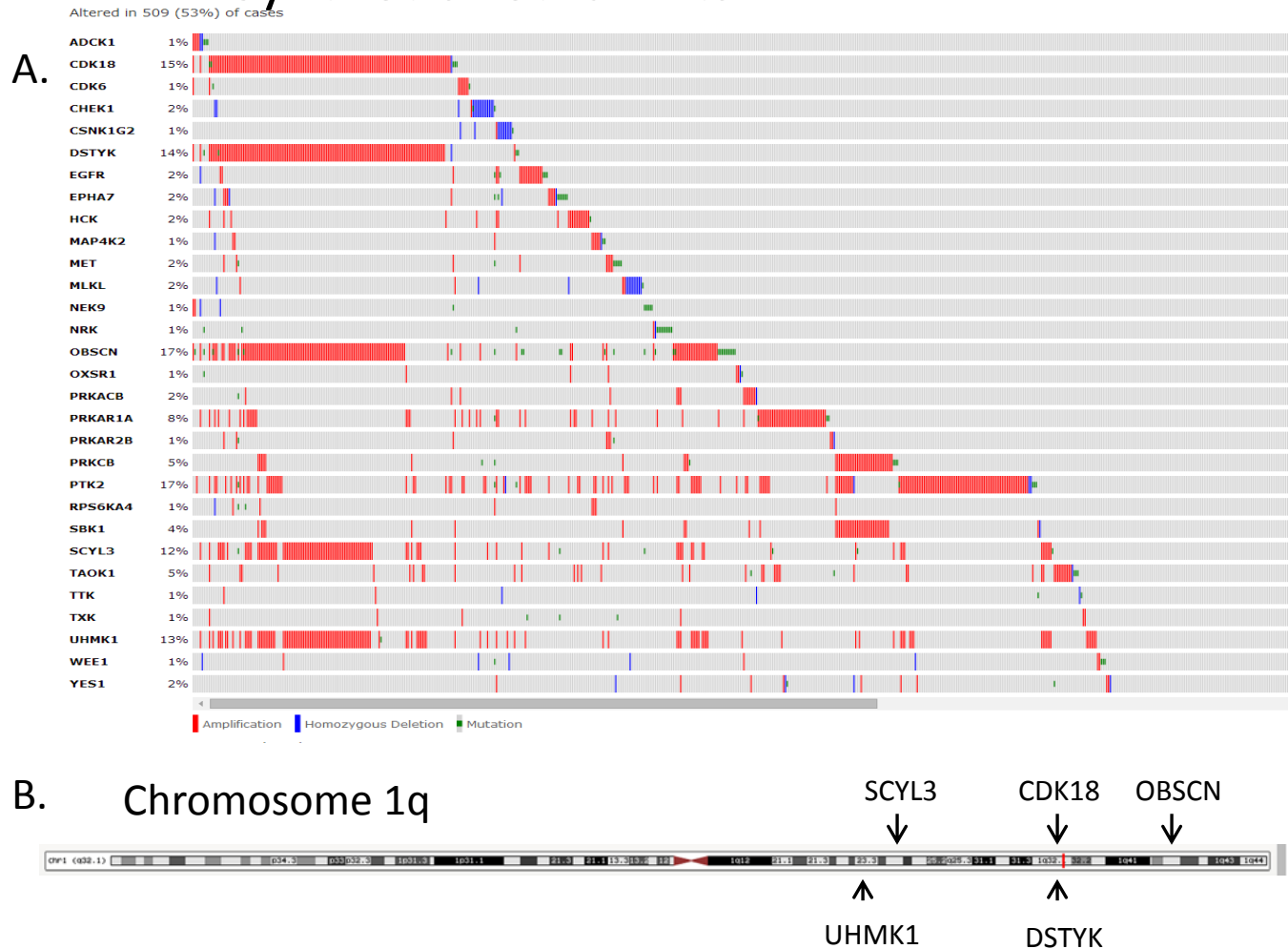
Figure 1. (A) The oncoprint of the synthetic lethal screen kinase hits under lactic acidosis in the TCGA breast cancer. (Red: Amplification, Blue: Deletion, Green: Somatic mutations). (B) 5 kinase hits (name indicated) are clustered in the regions of chromosomal 1q.
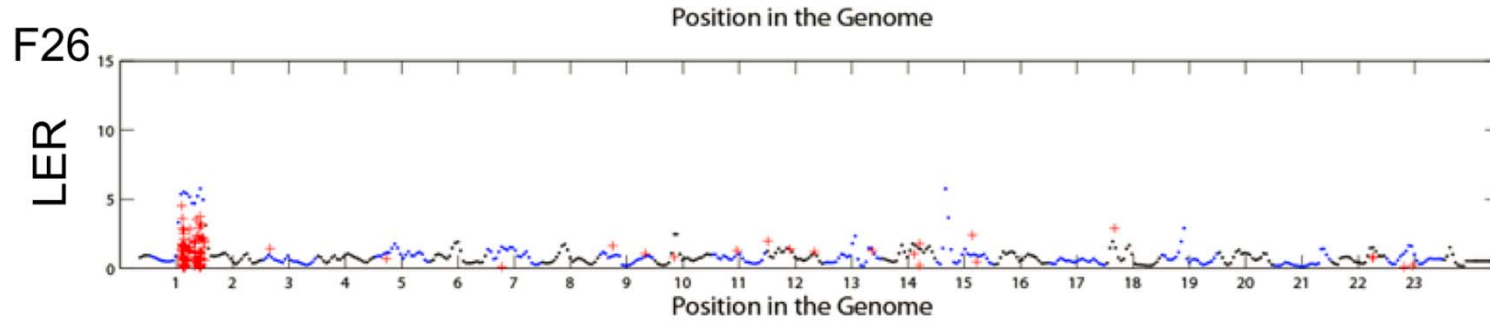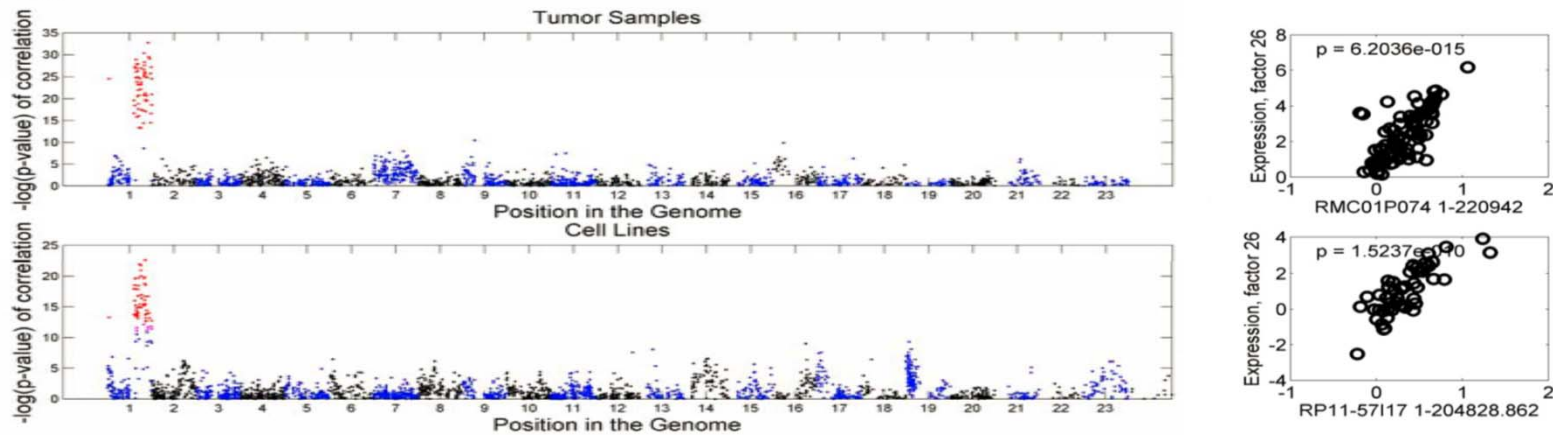
Figure 2. (A) Local enrichment ratios (y-axis) for the genes in latent factor 26 along the 23 autosomes (X-axis). Chromosomes are colored alternately blue and black for visualization. The red crosses designate the physical locations of the genes in the respective factor with vertical jitter added to allow visualization. (B) The degree of association (−log (p value) of Pearson correlation, Y-axis) between the expression of factor 26 with the BAC clones along the 23 chromosomes (X-axis) in breast tumors,(upper) , and cancer cell lines, (lowers), with the significant association with indicated BAC clones in 1q.
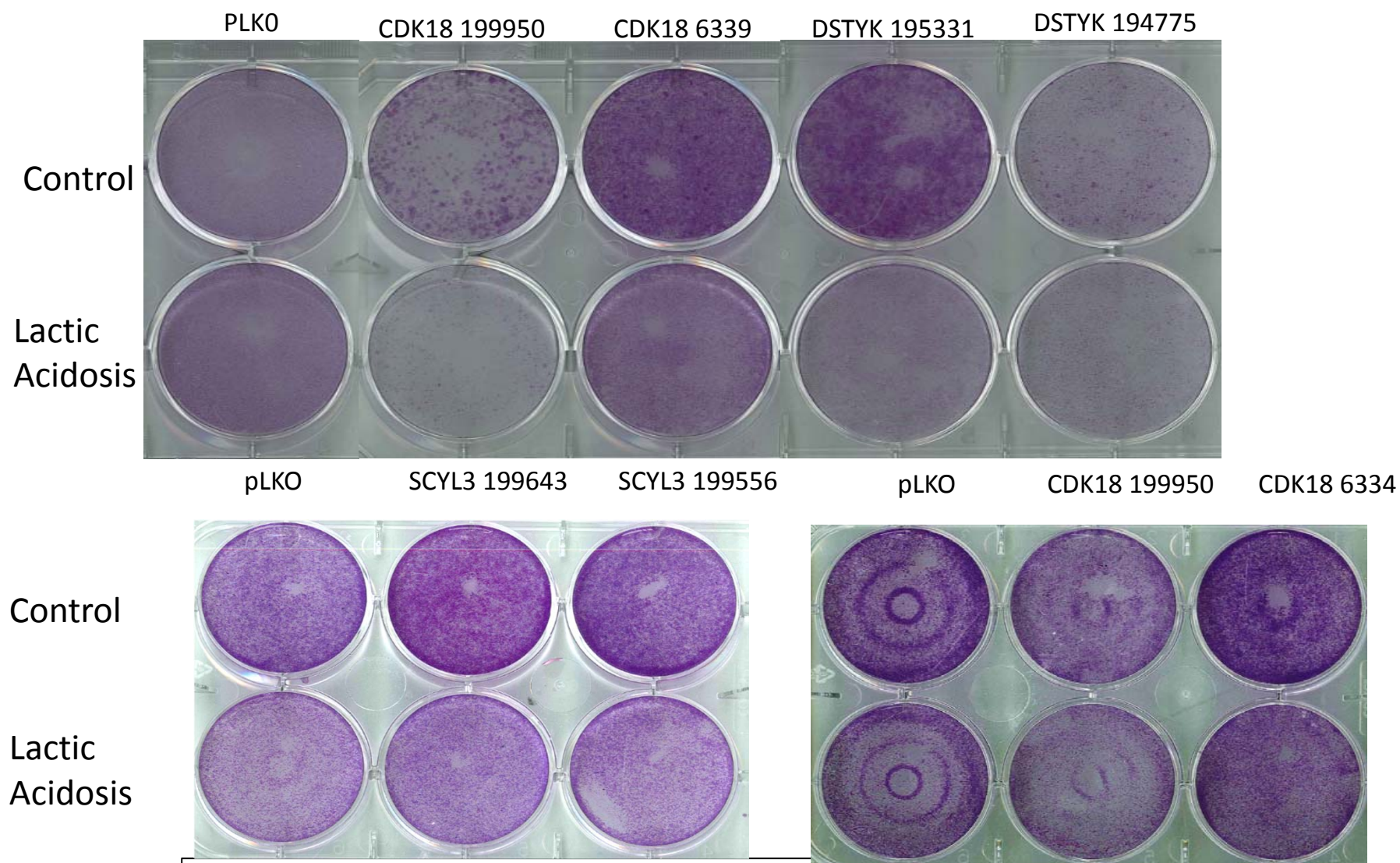
Figure 3. (A) Crystal Violet of MDA-MB-231 transduced with indicated shRNAs under control or lactic acidosis (25 mM lactate and pH 6.7) for 10 days.
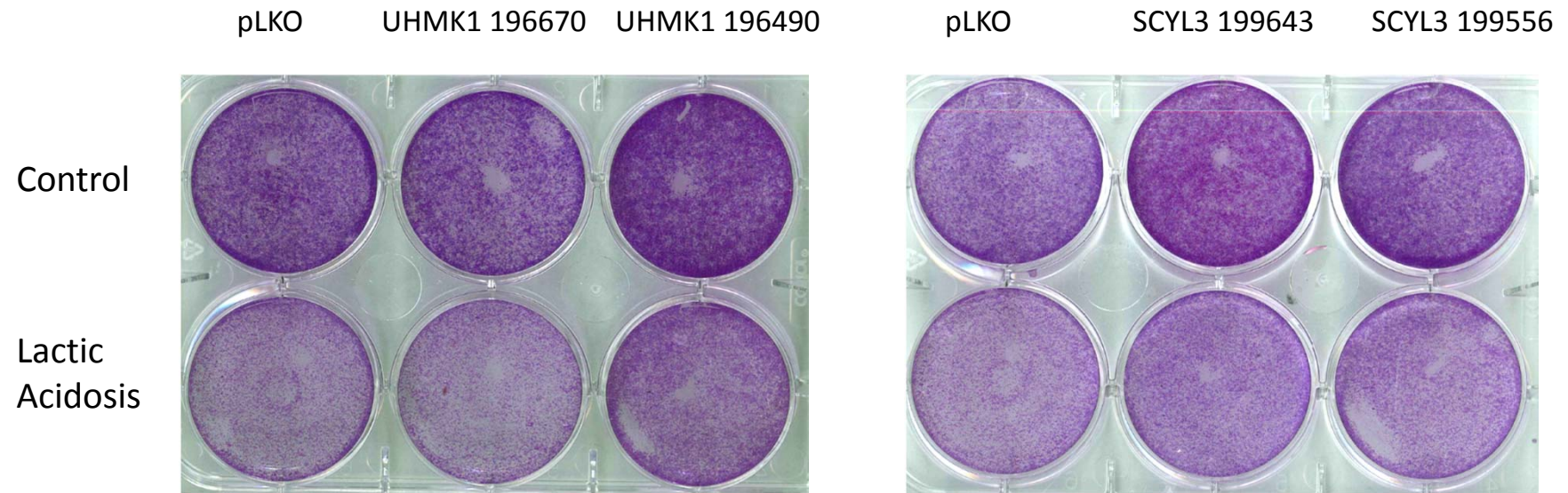
**Figure 4**. (A) Crystal Violet of MDA-MB-231 transduced with indicated shRNAs under control or lactic acidosis (25 mM lactate and pH 6.7) for ten days.
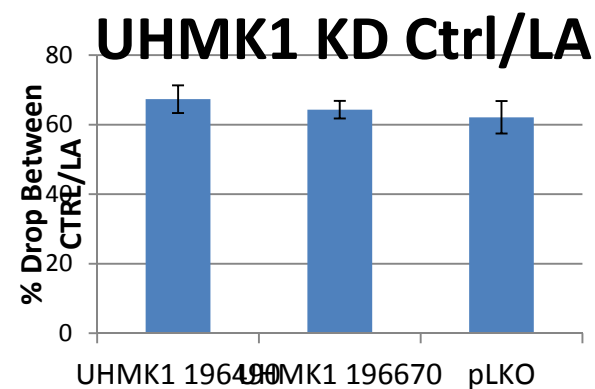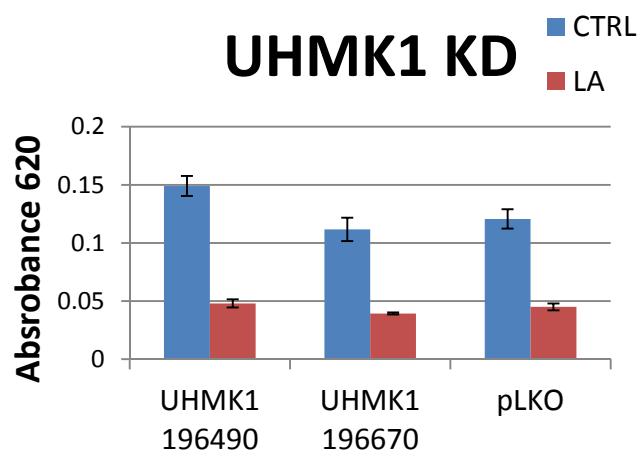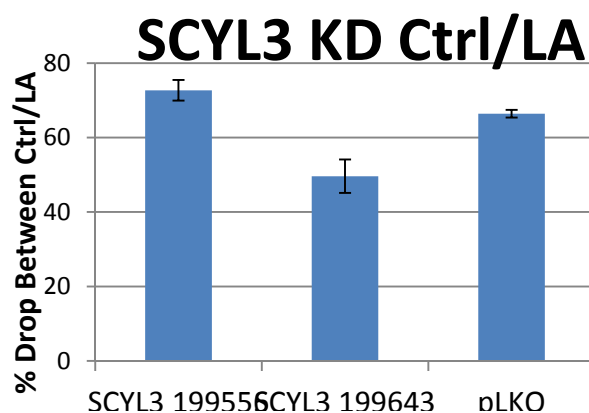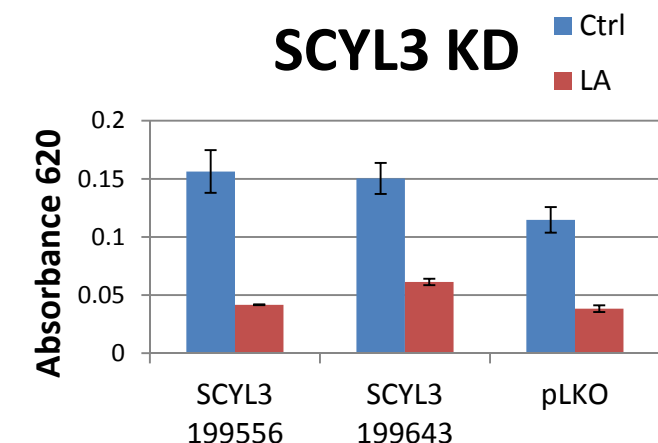
Figure 5. (A) Crystal Violet of MDA-MB-231 transduced with indicated shRNAs under control or lactic acidosis (25 mM lactate and pH 6.7) for ten days. The left panel showed the absorbance and right panels showed the ratios between Control over LA (Ctrl/LA) for each shRNAs.
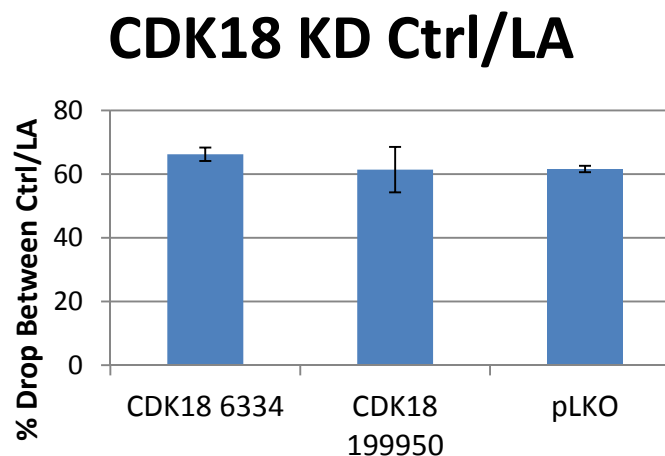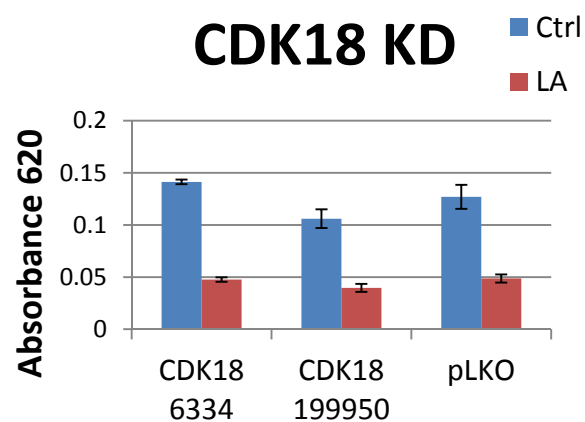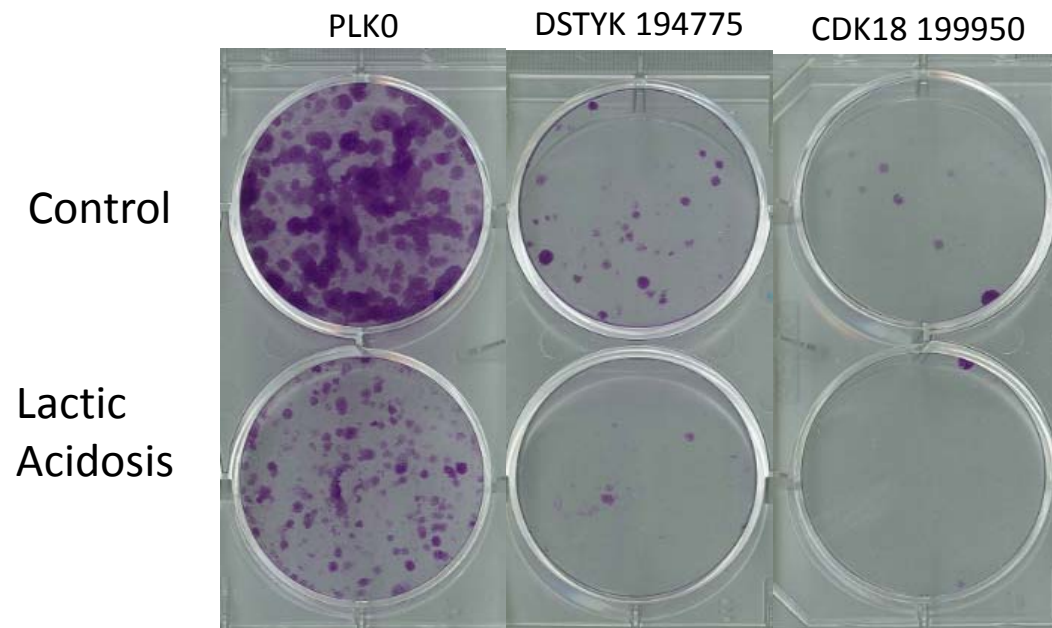
Figure 6. (A) Crystal Violet of MDA-MB-231 transduced with indicated shRNAs under control or lactic acidosis (25 mM lactate and pH 6.7) for 15 days.
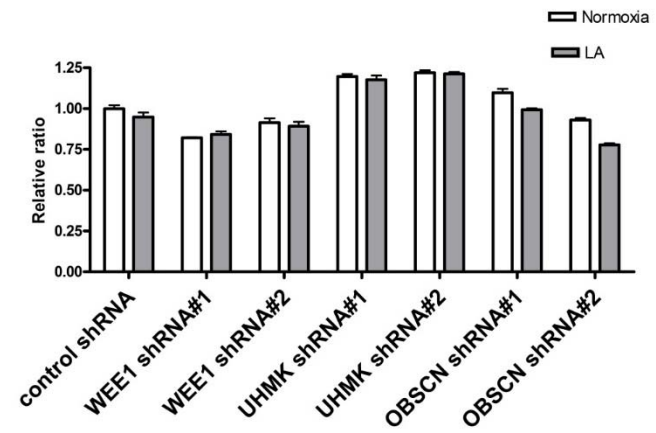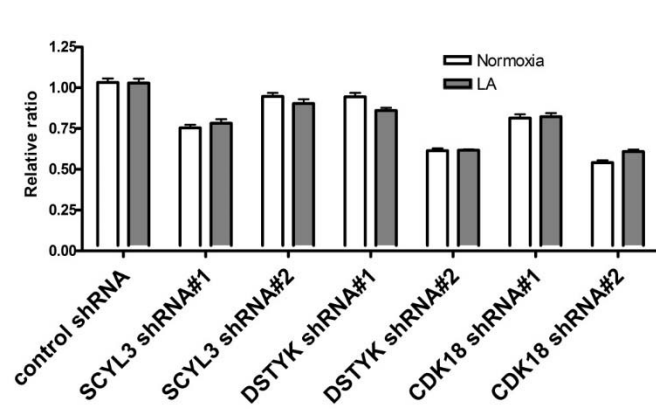
Figure 7. Cell Titer Glow of MDA-MB-231 transduced with indicated shRNAs under control or lactic acidosis for 3 days.
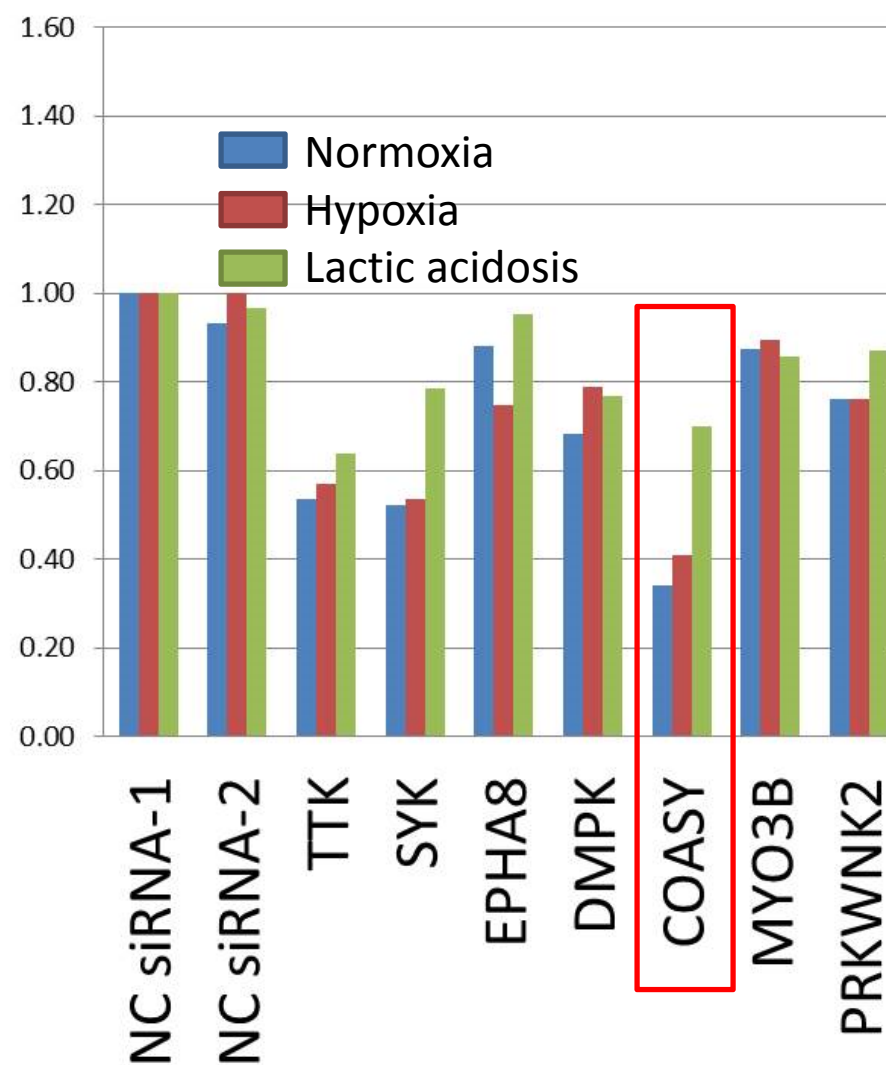
Figure 8. Cell Titer Glow of MDA-MB-231 transduced with indicated shRNAs under control or lactic acidosis for 3 days.
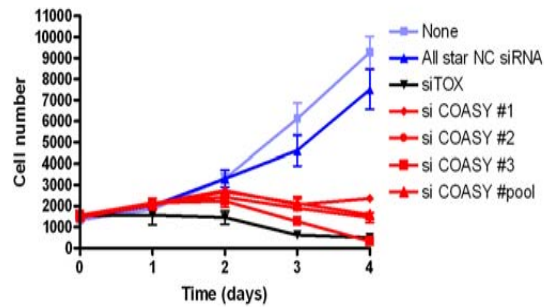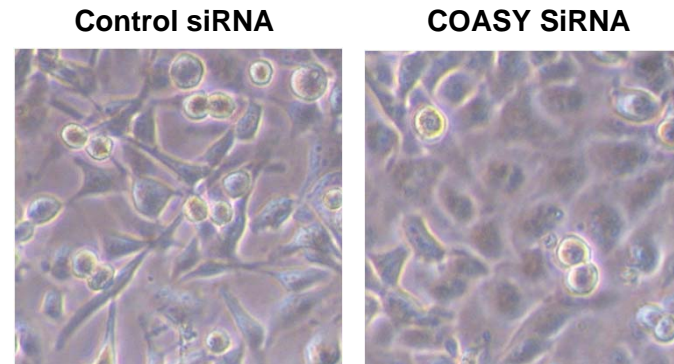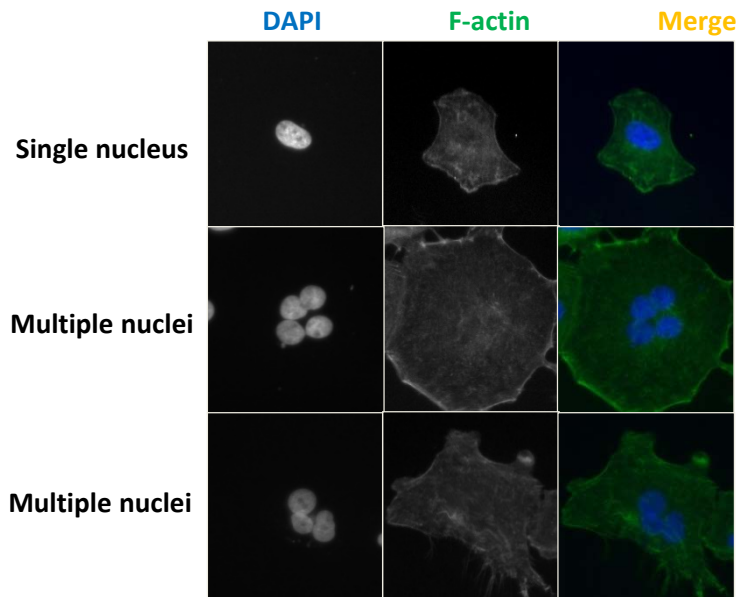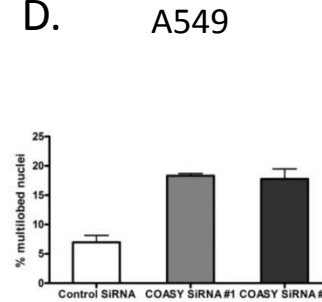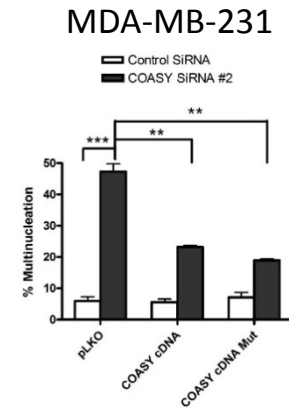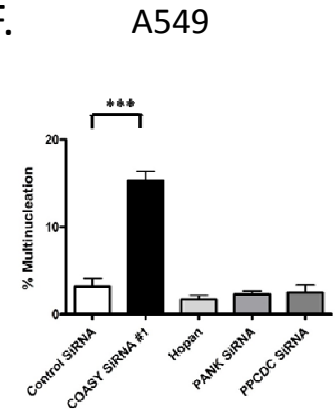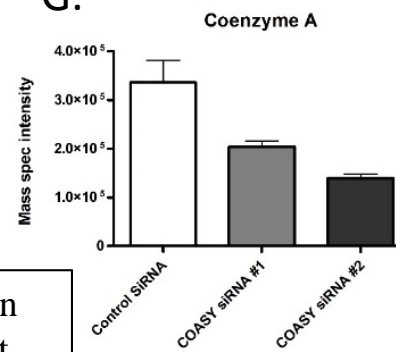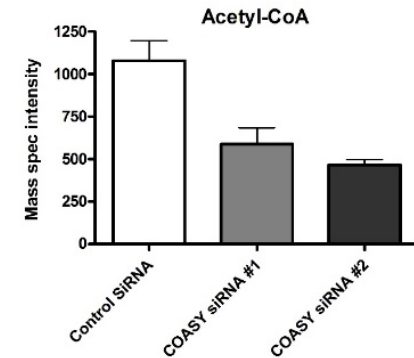
Figure 9. The silencing of COASY lead to multinucleation phenotypes which can be rescued by wild type and mutant COASY, suggesting an enzymatic independent function.
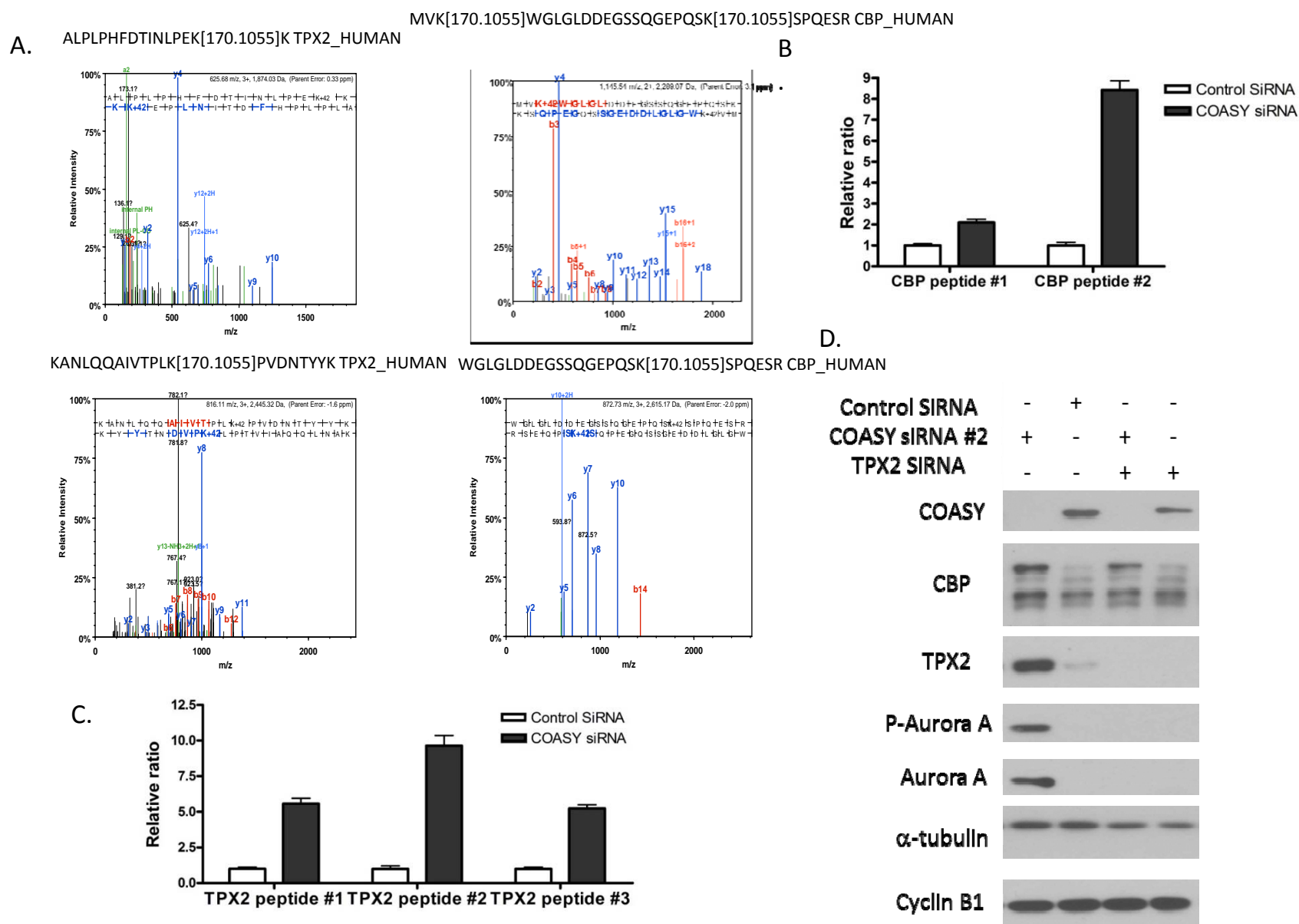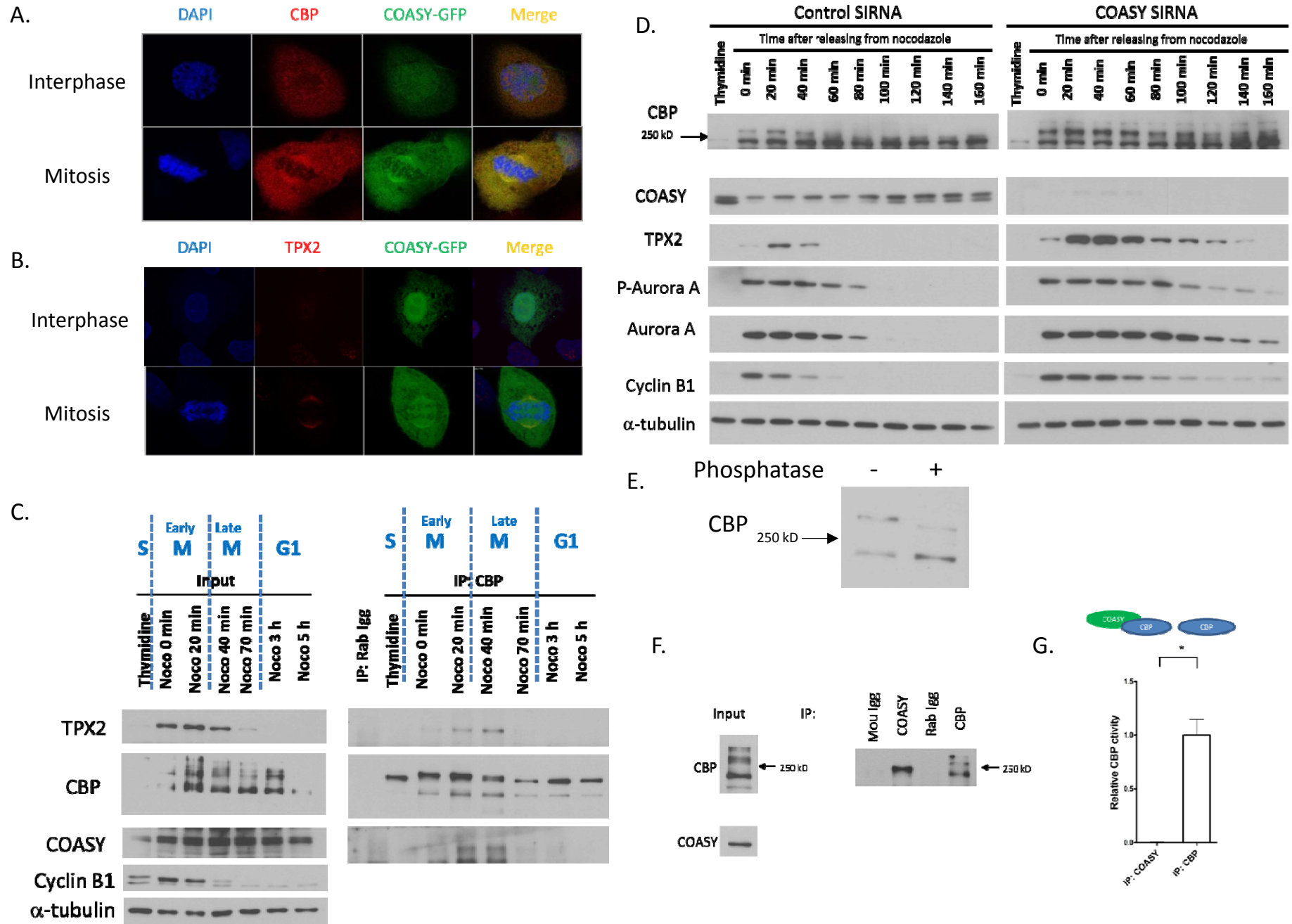
Fig 10. Mass spec revealed the acetylated proteins under COASY silencing during mitosis

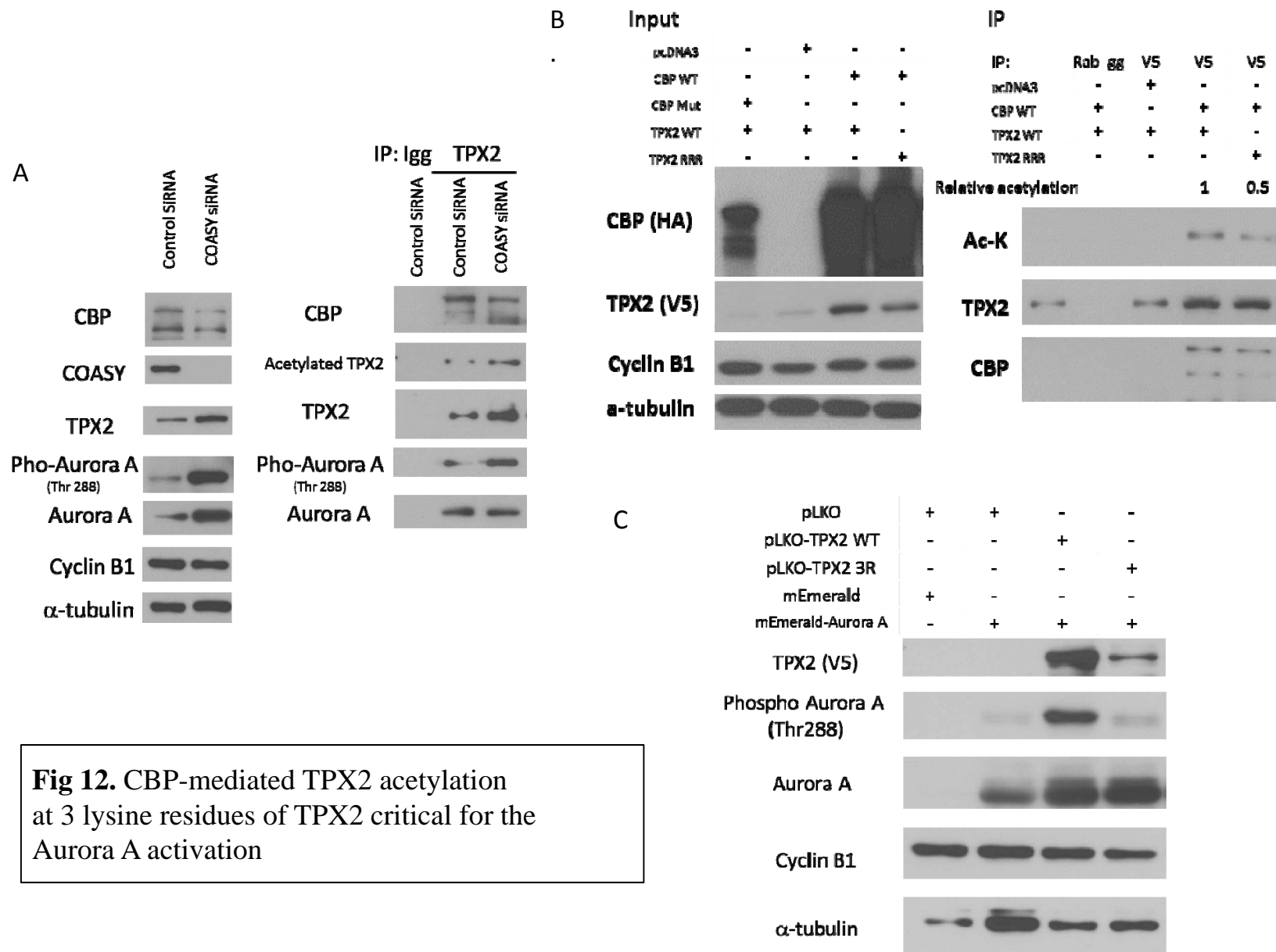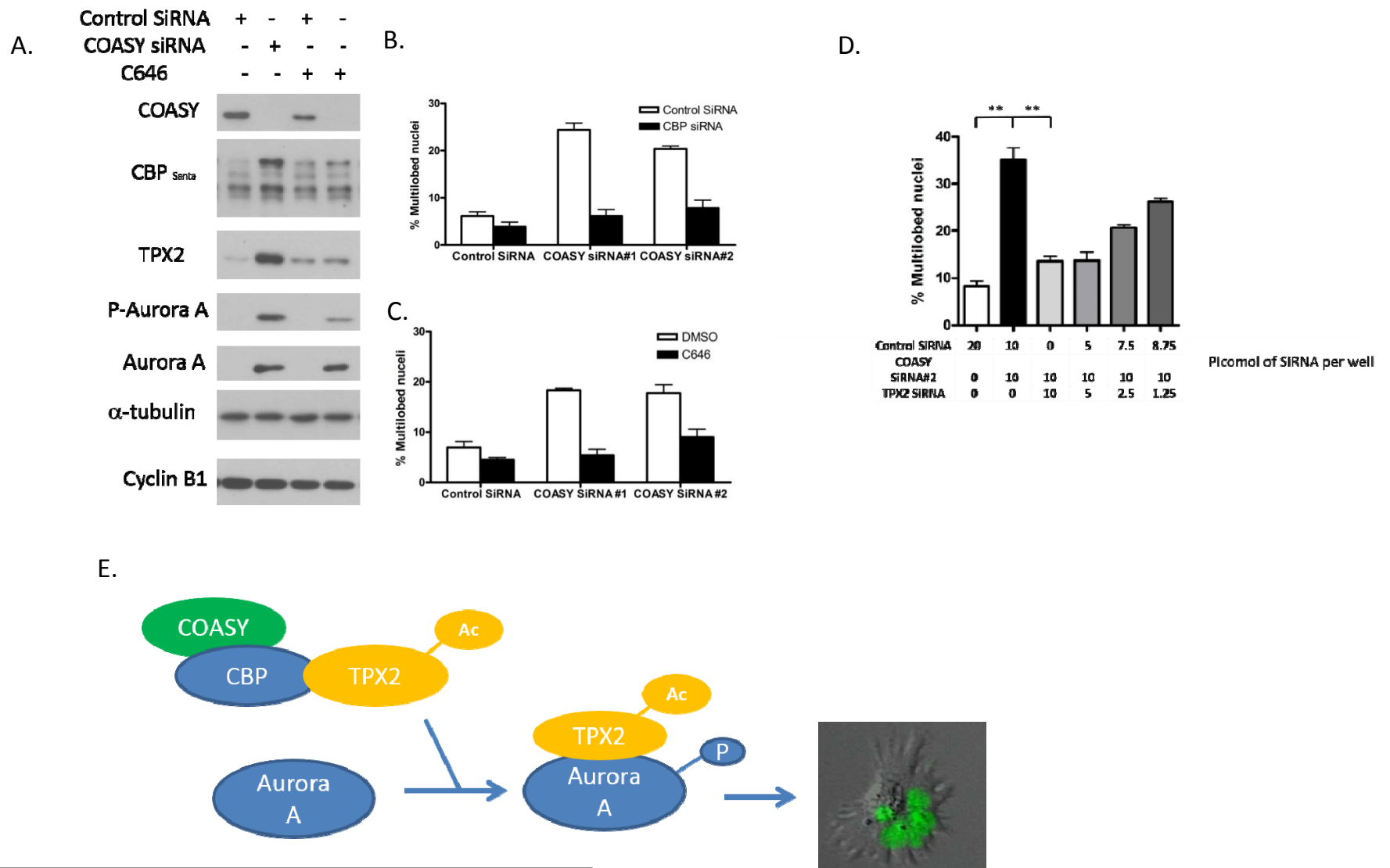**Fig 11.** COASY-CBP-TPX2 form a complex during early mitosis

Fig 12. CBP-mediated TPX2 acetylation at 3 lysine residues of TPX2 critical for the Aurora A activation

Fig 13. CBP silencing or inhibition rescues COASY dependent multinucleation

Figure 14. Cas9 nuclease is <u>directed</u> by <u>a</u> gRNA to complementary sequences in the genome, which <u>are</u> then cleaved and repaired by error-prone NHEJ. Taken from Mali et al Science, 2013.
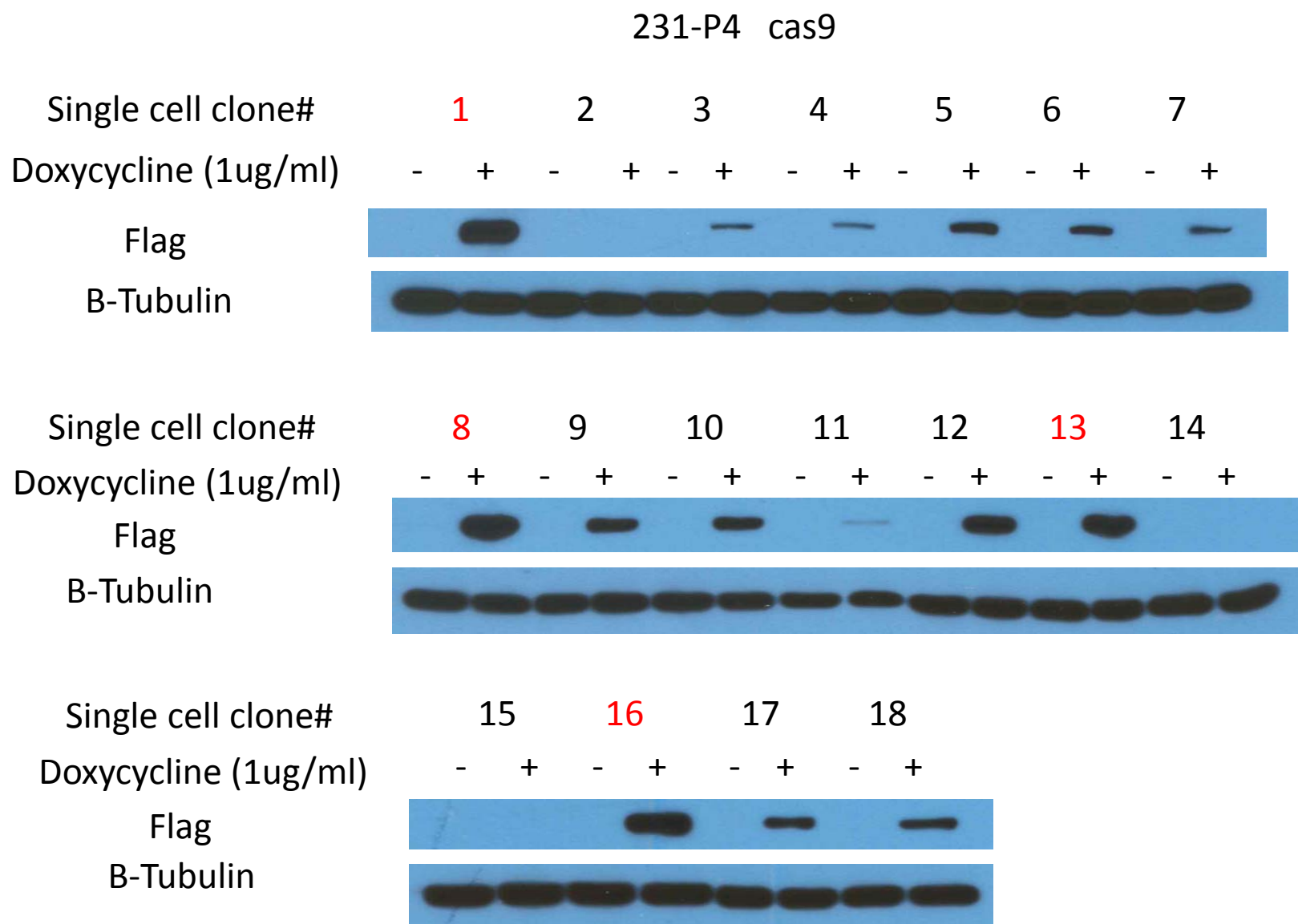
Fig 15 Selection of multiple MDA-MB231 cells with inducible Cas9. The number indicate each single cell clones with the best hits indicated in **red**.
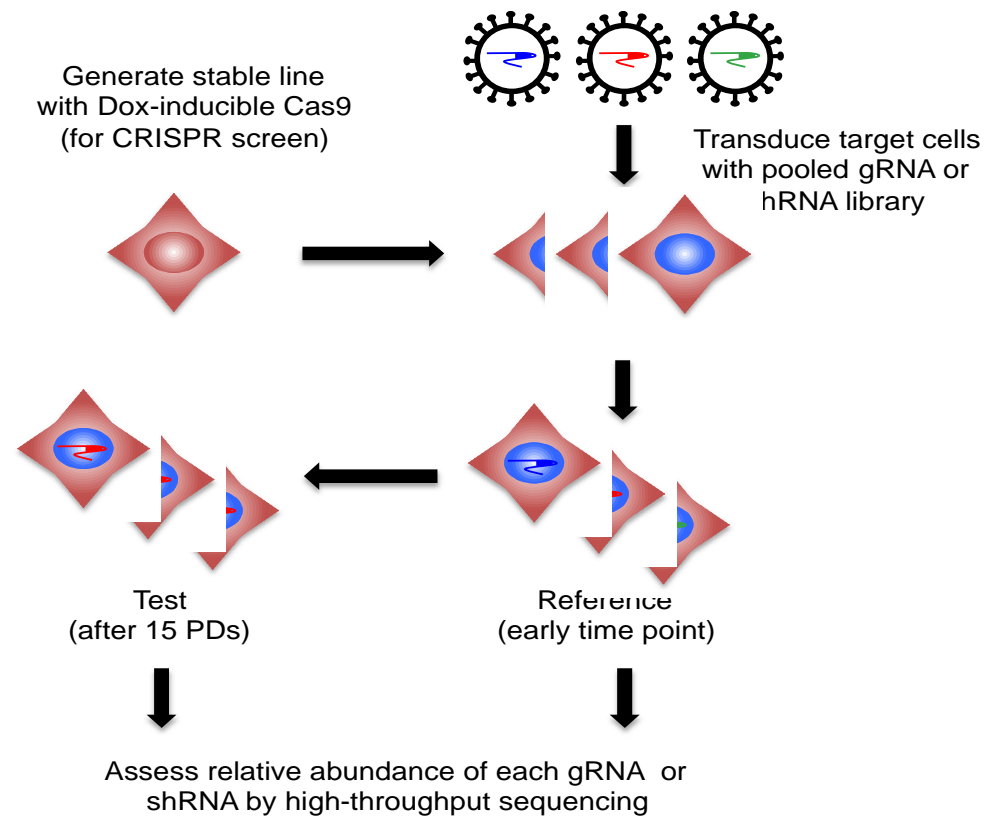
Generate stable line
with Dox-inducible Cas9
(for CRISPR screen)

Transduce target cells
with pooled gRNA or
hRNA library

Test
(after 15 PDs)

Reference
(early time point)

Assess relative abundance of each gRNA or
shRNA by high-throughput sequencing

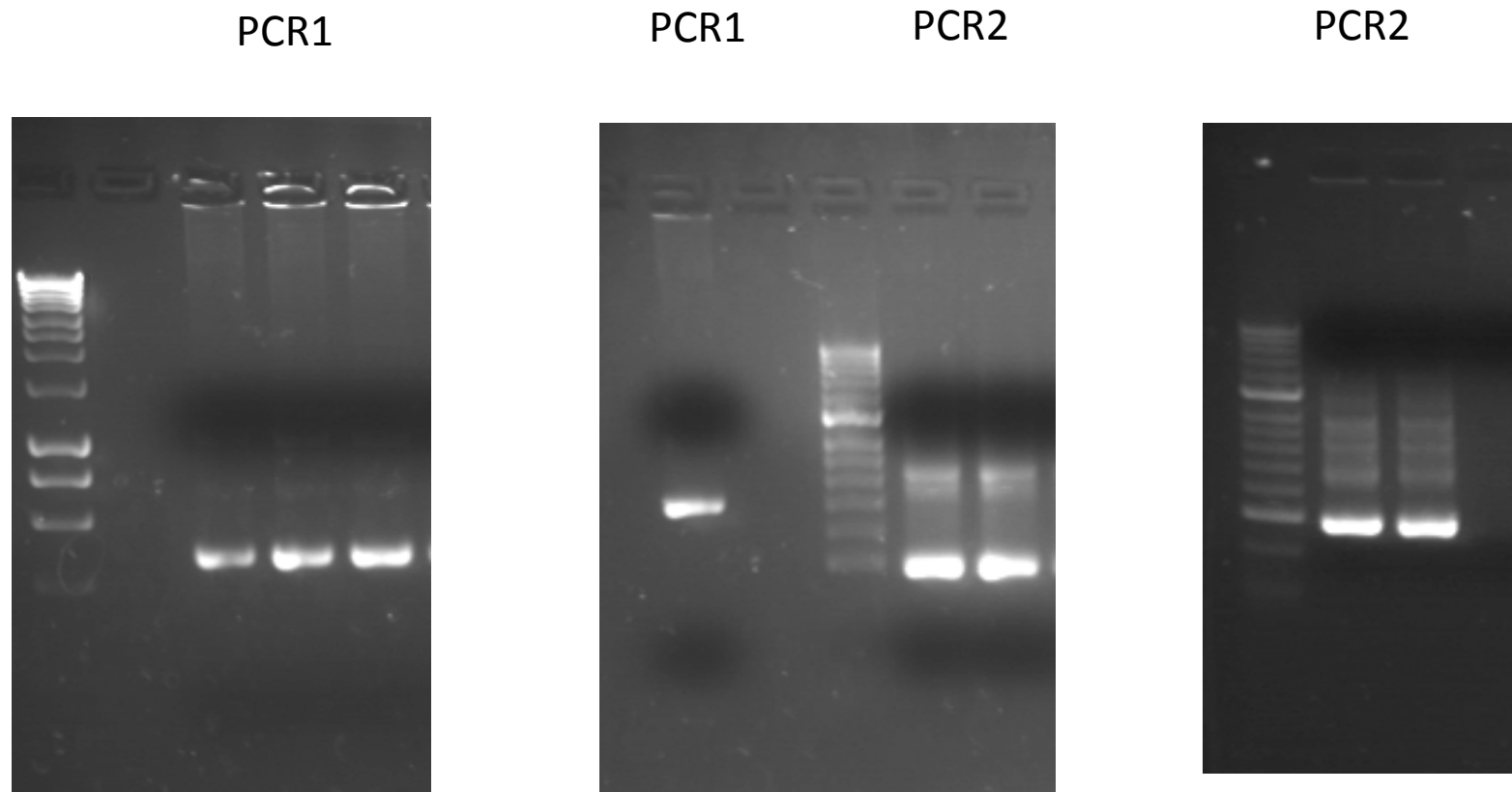**Fig 16**. Schematic overview of pooled shRNA or CRISPR gRNA library screening process

Fig 17. Amplification of the sgRNAs using two step PCR from the library.
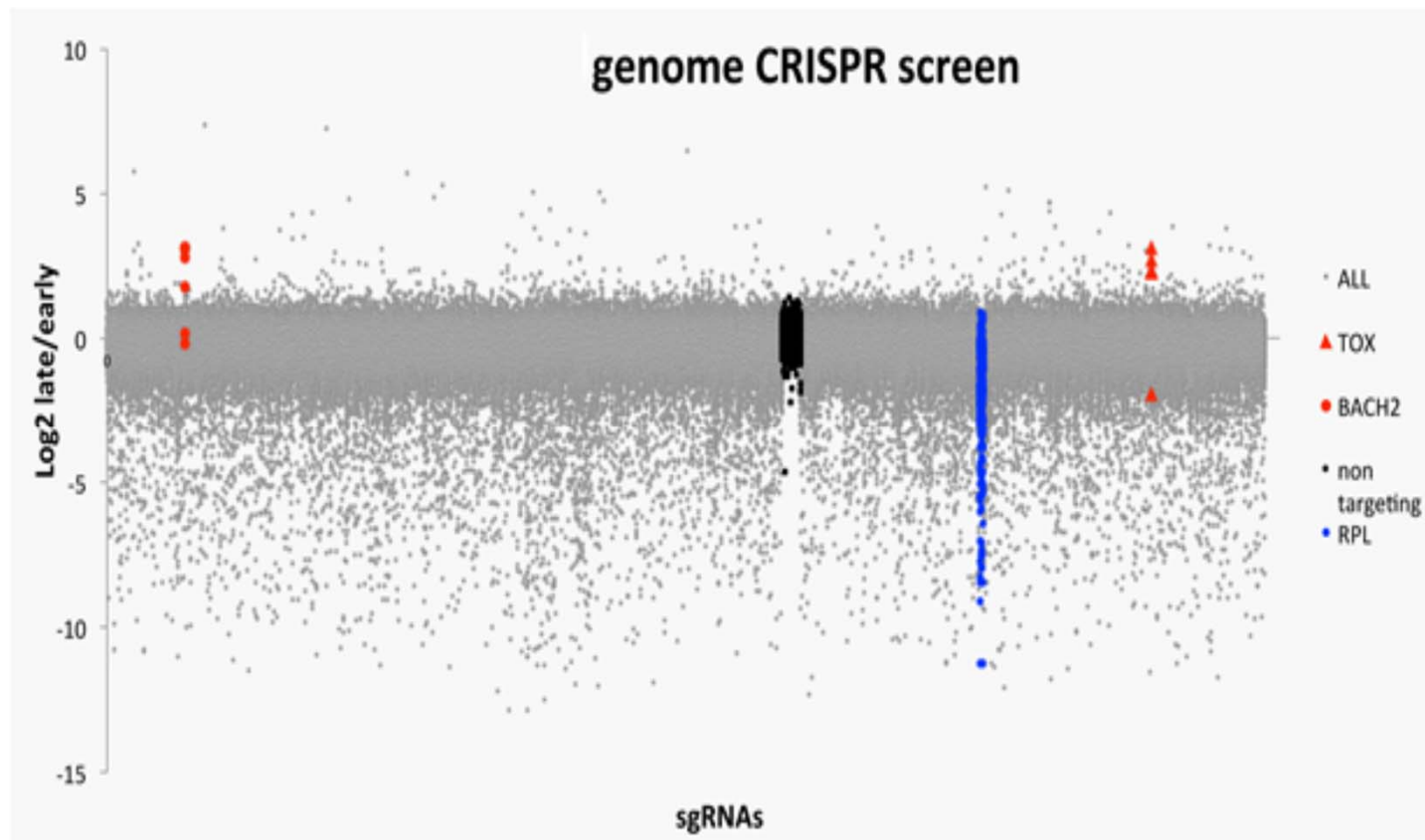
Fig 18 Fold change of each sgRNA in the GeCKO screen. Representative genes (TOX, BACH2), gene sets (RPL=large ribosomal subunit genes) and controls are shown as described to provide examples of depletion and enrichment.